Effects of Intermittent Parathyroid Hormone 1–34 Administration on Circulating Mesenchymal Stem Cells in Postmenopausal Osteoporotic Women

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Background: Intermittent parathyroid hormone (PTH) 1–34 administration stimulates osteogenesis and increases bone marrow mesenchymal stem cell (MSC) density; however, its effect on the circulating MSCs is unknown. This study aimed to examine the effect of intermittent PTH 1–34 administration on circulating MSCs in the peripheral blood of postmenopausal osteoporotic women.

Material/Methods: Fifty-four postmenopausal osteoporotic women at high risk of fracture were enrolled and administered either teriparatide (PTH 1–34) or alendronate for 12 months. Whole blood samples were obtained at baseline, 1, 3, 6, and 12 months after initiation of treatment. Flow cytometry analyses were performed to identify circulating MSCs (CD73+, CD90+, CD105+, CD34-, and CD45-). Serum markers of bone formation, bone resorption, as well as bone mineral density (BMD) were serially measured. Circulating MSCs were isolated from peripheral blood of teriparatide treated women and cultured in osteogenic medium to examine their osteogenic differentiation potential.

Results: Teriparatide treatment increased circulating MSCs to 141±96% (*P*<0.001) by month 1, persisting until month 12; this increase was positively associated with increases in bone formation and bone resorption biomarkers (at month 6) and spine BMD (at month 12). Furthermore, intermittent PTH 1–34 administration promoted *in vitro* osteogenic differentiation of circulating MSCs, evident from increased alkaline phosphatase (ALP) activity, ALP-expressing cell density, calcium deposition, and Runx-2, OSX, COL 1a1, and osteocalcin mRNA upregulation.

Conclusions: Intermittent PTH 1–34 administration increased circulating MSC density in women with postmenopausal osteoporosis and enhanced *in vitro* osteogenic differentiation potential of these cells.

MeSH Keywords: Alendronate • Mesenchymal Stromal Cells • Osteoporosis, Postmenopausal • Parathyroid Hormone

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Background

Maintenance of the mechanical integrity of bones depends on the homeostasis of osteogenesis and the homeostatic bone resorption. An imbalance between these phenomena is the primary contributor of osteoporosis, characterized by low bone mass, consequent occurrence of fractures resulting from bone fragility, and compromised fracture healing [1]. The rate and quality of osteogenesis depends on the density and activity of osteoblasts primarily originating from mesenchymal stem cells (MSCs). Although bone marrow is well accepted as the primary source of these precursor cells, MSCs can be isolated from peripheral blood (PB-MSCs) [2,3], also referred to as circulating MSCs [4]. MSC density is reportedly significantly reduced and their osteogenic differentiation potential is markedly diminished in the bone marrow of women with postmenopausal osteoporosis, thereby significantly decreasing osteogenesis [5–7].

Osteoporosis treatment predominantly involves anti-resorptive agents, such as bisphosphonates, which suppress osteoclast-mediated bone resorption [8]. Another therapeutic alternative, especially for severe cases, is teriparatide, a synthetic and active fragment of parathyroid hormone (PTH), comprising N-terminal amino acid residues 1–34 (PTH 1–34) [9,10]. PTH is considered the primary regulator of calcium homeostasis and reportedly stimulates bone formation and bone resorption and can increase or decrease bone mass, depending on the administration mode [11–13]. Daily subcutaneous injections of PTH stimulates more osteogenesis than bone resorption by directly acting on osteoblasts to promote osteoblastogenesis and reduce osteoblast apoptosis [12,14,15]. Furthermore, another mechanism underlying the anabolic effect of PTH is the stimulation of proliferation and differentiation of bone marrow MSCs into the osteoblast lineage upon short-term administration [16,17]. In some animal studies, PTH promoted exogenous migration of bone marrow MSCs to fracture sites, but also their terminal differentiation to an osteogenic lineage [18,19].

Although the effect of PTH on MSCs recruitment has been widely studied in animal models over past decades, limited information is available regarding its physiological effects on circulating MSCs from human clinical studies. In this study, we hypothesized that intermittent PTH 1-34 administration increases circulating MSC density in women with postmenopausal osteoporosis and enhances their osteogenic differentiation *in vitro*.

Material and Methods

Study population

This 12-month, open-label, randomized controlled study involved 54 women with postmenopausal osteoporosis with a

high risk of fracture, referred to our center between October 2015 and November 2017. Women were eligible for enrollment in the study if they were age older than 50 years, ambulatory, a period of at least 3 years had elapsed since menopause, and they had a high risk of fracture. High risk of fracture was defined in accordance with the following criteria: T score ≤–2.5 at the spine, hip, or femoral neck; T score ≤–2.0 with at least 1 bone mineral density (BMD)-independent risk factor (fracture after age 50 years, parental hip fracture after age 50 years, previous history of hyperthyroidism, inability to get up from a chair with arms raised, or current smoking); or T score ≤–1.0 with history of fragility-related fracture [20]. The exclusion criteria were unhealed bone fractures (x-ray and MRI were performed to exclude morphometric vertebral fractures, using Genant semi-quantitative method [21] and the malignant diseases of the vertebrae were also excluded); 25-hydroxyvitamin D concentration in serum lower than 20 ng/mL (50 nmol/L), hyperparathyroidism, hyperthyroidism, hypercalcemia, congenital or acquired bone disease, and any other disease causing secondary osteoporosis; history of malignant disease or radiation therapy, anemia, severe cardio-pulmonary, liver, renal, or major psychiatric disease, and excessive alcohol intake. Women were also excluded if they had been treated with bisphosphonates for a total of more than 12 months, or they had taken oral bisphosphonates or glucocorticoids within 6 months before enrollment, estrogen, selective estrogen-receptor modulators, or calcitonin within 3 months before enrolment, or they had received intravenous bisphosphonates, teriparatide, PTH, or strontium ranelate. This study was prospectively approved by the Medical Ethical Committee of Tianjin Medical University General Hospital and all participants provided written informed consent.

Study design

Fifty-four patients were randomly divided into 2 groups receiving either teriparatide (Forteo®, Eli Lilly and Co., Indianapolis, IN, USA) or alendronate (Fosamax®, Merck & Co., Kenilworth, NJ, USA) therapy for 12 months (Table 1). The teriparatide group (n=28; age, 61.7±6.6 years) received teriparatide 20 μg once daily via a self-administered subcutaneous injection and the alendronate group (n=26; age, 62.8±7.3 years) received oral alendronate 70 mg tablet once weekly. In both groups, to minimize the risk of hypercalcemia or hypercalciuria, patients received daily supplements of calcium (1 g/day) and vitamin D (800 IU/day). Patients visited our clinic at 0, 1, 3, 6, and 12 months. After a rest of 20 minutes, whole-blood samples were collected from all participants, from a superficial brachial vein, with care to avoid stasis, hemolysis, and contamination by tissue fluids or exposure to glass. Flow cytometry analysis was performed to determine the number of circulating MSCs.

Six patients were randomly selected from the 28 women in the teriparatide group for collection of peripheral blood samples

Table 1. Demographics and baseline characteristics of patients.

	Teriparatide group (n=28)	Alendronate group (n=26)	*P* value
Age (years)	61.7±6.6	62.8±7.3	0.554
Height (cm)	160.9±6.2	161.4±5.1	0.786
Weight (kg)	63.9±8.3	64.8±5.2	0.622
Body mass index (kg/m^2)	24.5±2.2	24.8±1.3	0.564
Serum parameters			
25-Hydroxyvitamin D (ng/mL)	32.5±6.7	33.0±8.9	0.807
β-CTX (ng/mL)	0.38±0.12	0.38±0.11	0.948
PINP (μg/L)	46.8±15.2	47.1±13.6	0.957
Osteocalcin (ng/mL)	51.2±15.8	54.5±14.3	0.434
DXA BMD (g/cm^2)			
Posterior-anterior lumbar spine	0.852±0.076	0.845±0.078	0.746
T score	−1.8±0.7	−2.0±0.9	
Femoral neck	0.640±0.066	0.624±0.070	0.394
T score	−1.9±0.8	−2.0±1.0	
Total hip	0.779±0.074	0.762±0.076	0.421
T score	−1.6±0.6	−1.6±0.7	
Circulating MSCs (/uL)	0.402±0.160	0.424±0.185	0.617

Values are means ±standard deviation.

at baseline (control group) and 1 month (PTH group) for isolating circulating MSCs and evaluating their osteogenic differentiation potential.

Flow cytometry analyses

Whole-blood samples were collected from all participants and drawn into EDTA-anticoagulated tubes (Sarstedt, Nümbrecht, Germany) immediately. For each participant, 200 μL of peripheral blood was stained with fluorescence-conjugated mouse anti-human monoclonal antibodies. The antibodies used for staining were phycoerythrin (PE)-conjugated anti-CD73, fluorescein isothiocyanate (FITC)-conjugated anti-CD105, peridinin chlorophyll protein (PerCP)-conjugated anti-CD90, phycoerythrin-cyanine 7 (PC7)-conjugated anti-CD45, and allophycocyanin (APC)-conjugated anti-CD34 (all obtained from Beckman Coulter, Marseille, France). For each sample, an isotype control was prepared to monitor background staining using mouse anti-human CD45-PC7 negative control in conjunction with mouse IgG1 fluorescence-labeled antibodies IgG1-PE, IgG1-FITC, IgG1-PerCP, and IgG1- APC (all obtained from Beckman Coulter). Staining was performed according to the manufacturer's instructions. Following staining, samples and isotype controls were lysed to remove the red cells and fixed by paraformaldehyde. Immediately after fixing, samples and isotype controls were subjected to flow cytometry using a FACSCanto™ II cytometer (BD Biosciences, San Jose, CA, USA) and analyzed by and the FACSDiva™ Software (BD Biosciences, version 6.1.3). The population of MSCs was identified, as cells positive for CD73-PE, CD90-PerCP, and CD105-FITC, and cells negative for CD45-PC7 and CD34-APC. The percentage of identified circulating MSCs relative to the total number of live cells was calculated for each sample and further normalized by subtracting the percentage of the relevant isotype control.

Measurements of bone turnover and BMD

Fasting blood samples were collected in the morning and stored at -50°C until they were assayed for intact N-terminal propeptide of type I collagen (PINP), osteocalcin (OC), and β-C-telopeptide of type I collagen (β-CTX) in our laboratory, using electrochemiluminescence measurement techniques with a fully automated system (cobas® e 411 immunoanalyzer, Roche Diagnostics Corp., Indianapolis, IN, USA) [22,23]. BMD was measured in all patients by dual-energy x-ray absorptiometry scan (SONOST-3000; OsteoSys Co., Ltd., Seoul, Korea) at the posterior-anterior lumbar spine (PA spine), total hip and femoral neck.

Isolation, culture, and immunophenotypic identification of circulating MSCs

Peripheral blood was sampled from the 6 patients before initiation of PTH 1-34 treatment (control group) and 1 month after treatment (PTH group). Each blood sample was diluted immediately with heparinized saline in a 1: 1 ratio. The diluted blood was gently loaded into an equivalent volume of Ficoll solution (1.077 g/mL; Dingguo Changsheng, Beijing, China), and subjected to density-gradient centrifugation at 2500 rpm for

20 minutes at 37°C. The mononuclear cell fraction was harvested and rinsed twice with Hank's Balanced Salt Solution (HBSS) and then cultured at a density of 10^6 cells/cm^2 in α-MEM (Invitrogen, Carlsbad, CA, USA) supplemented with 15% fetal bovine serum (FBS; Gibco, New York, NY, USA), 100 U/mL penicillin, 100 µg/mL streptomycin, and 2 mM glutamine. The medium was replaced every 3 days to eliminate non-adherent cells, and adherent cells were passaged regularly until approximately 80–90% confluence. Circulating MSCs at the 3rd passage (P3) were used for further experiments. Flow cytometric analysis was performed to identify the immunophenotypic features of P3 circulating MSCs.

Osteogenic differentiation

To assess osteogenic differentiation, circulating MSCs at P3 were seeded at 5×10^4 cells/well and cultured in basic medium for 24 hours and then subjected to osteogenic induction medium (OIM) comprising α-MEM medium supplemented with 10 mM β-glycerol phosphate, 0.1 µM dexamethasone, and 0.2 mM ascorbic acid. The medium was changed every 3 days after initial plating.

RNA extraction and quantitative real-time PCR analysis

Total RNA was extracted using TRIzol Reagent (Invitrogen) in accordance with the manufacturer's instructions on day 0, 7, 14, and 21 after osteogenic induction. The mRNA expression of growth factor collagen type 1 (Col 1a1), osteocalcin (OC), and transcriptional factors Osterix (OSX) and runt-related transcription factor 2 (Runx-2) were examined via quantitative real-time PCR. For reverse transcription of mRNA, random-primed cDNA was synthesized from 2 mg of total RNA using a PrimeScript RT reagent kit (TaKaRa, Dalian, China). Subsequent real-time PCR amplification was performed using 2 µL of cDNA product in a 25-µL reaction volume, using Step OnePlus™ Real-Time PCR System platform (ABI, Thermo Fisher Scientific, Waltham, MA, USA). The primers used in the study were designed using Primer Express 5.0 based on published cDNA sequences, as follows: COL 1a1, 5'-TTCCCGGTGAATTCGGTCTC-3' and 5'-ACCTCGGATTCCAATAGGACCAG-3'; OC, 5'-CCTAT TGGCCCTGGCCGCAC-3' and 5'-ACTGGGGCTCCCAGCCATTGA-3'; OSX, 5'-GCCTACTTACCCGTCTGACTTT-3' and 5'-CCCACTATTGCCAA CTGC-3'; Runx-2, 5'-GCACCCAGCCCATAATAGA-3' and 5'-TGG AGCAAGGAGAACCC-3'; GAPDH, 5'-GGCACAGTCAAGGC TGAGAATG-3' and 5'-ATGGTGGTGAAGACGCCAGTA-3'.

The PCR condition was the following: initial denaturation at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 30 seconds. All real-time PCR reactions were carried out in triplicate, and relative mRNA expression levels were normalized with that of *GAPDH* as the internal control, using the ΔΔCT method and are presented as expression fold-change relative to the control.

Assessment of alkaline phosphatase (ALP) activity and staining

To assess alkaline phosphatase (ALP) activity, MSCs were harvested on days 7, 14, and 21, washed with PBS, and lysed with a probe sonicator for 15 seconds in 2 mL buffer (50 mM [pH 7.2] Tris-HCl, 0.1% Triton X-100, and 2 mM MgCl$_2$). Sonicated cell lysates were then centrifuged for 10 minutes at 3500 rpm at 4°C, and the supernatants were used for the assays. ALP activity was determined using a commercial kit in accordance with the manufacturer's instructions (Nanjing Jiancheng Bioengineering Ltd., Nanjing, China) and data are expressed as nmol/15 min/ mg protein. For ALP staining, cells were fixed with 4% paraformaldehyde in PBS for 15 minutes and stained with substrate solution (0.1 mg/mL naphthol AS-MX phosphate, 0.6 mg/mL fast red violet LB salt in Tris buffer, pH 8.74) for 30–60 minutes at 37°C, and rinsed thrice with PBS to eliminate excess stain.

Alizarin Red S staining and calcium deposition volume assay

For Alizarin Red S staining, MSCs were harvested on days 7, 14, and 21, washed twice with PBS (pH 7.4), fixed with 10% formaldehyde for 10 minutes, washed thrice with distilled water, and then incubated with 40 mmol/L Alizarin Red S for 1 hour at 37°C. Thereafter, cultures were washed thrice with deionized water. Stained cultures were photographed, and calcium deposition volume was determined using a calcium colorimetric assay kit (Biovision, San Francisco, CA, USA) in accordance with the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using SPSS Statistics for Windows, Version 22 (SPSS, Inc., Chicago, IL, USA). Results were presented as mean ± standard deviation (SD) values. Within-group pairwise comparisons between the baseline and end-point values were performed using paired *t*-tests, and the percent change in each variable was compared between the control and PTH groups, using unpaired *t*-tests. Pearson's correlation analysis was performed to determine associations between changes in MSCs and bone-turnover markers and BMD. Significance was accepted at a *P* value of <0.05.
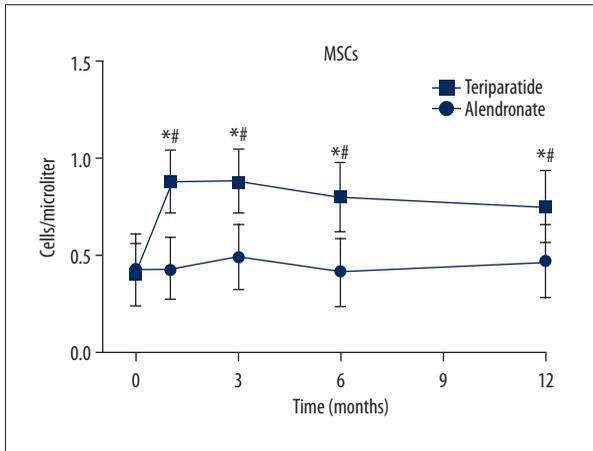
Figure 1. Circulating mesenchymal stem cells (MSCs) during 12 months of teriparatide and alendronate treatment. * *P*<0.05 versus baseline, # *P*<0.05 versus alendronate. Data presented are mean ±standard deviation.

Results

Characteristics of study patients

The baseline demographics of 54 patients are presented in Table 1. No patients dropped out of this study. There was no significant difference in baseline characteristics between the 2 groups.

Teriparatide treatment increased the number of circulating MSCs

MSCs were assessed via flow cytometry analysis at months 0, 1, 3, 6, and 12 after initiation of treatment and expressed as cell/μL of blood (Figure 1). In the teriparatide treatment group, the number of circulating MSCs increased by 141±96% (*P*=0.001) at month 1, 140±86% (*P*=0.003) at month 3, 122±99% (*P*=0.004) at month 6, and 108±95% (*P*=0.014) at month 12
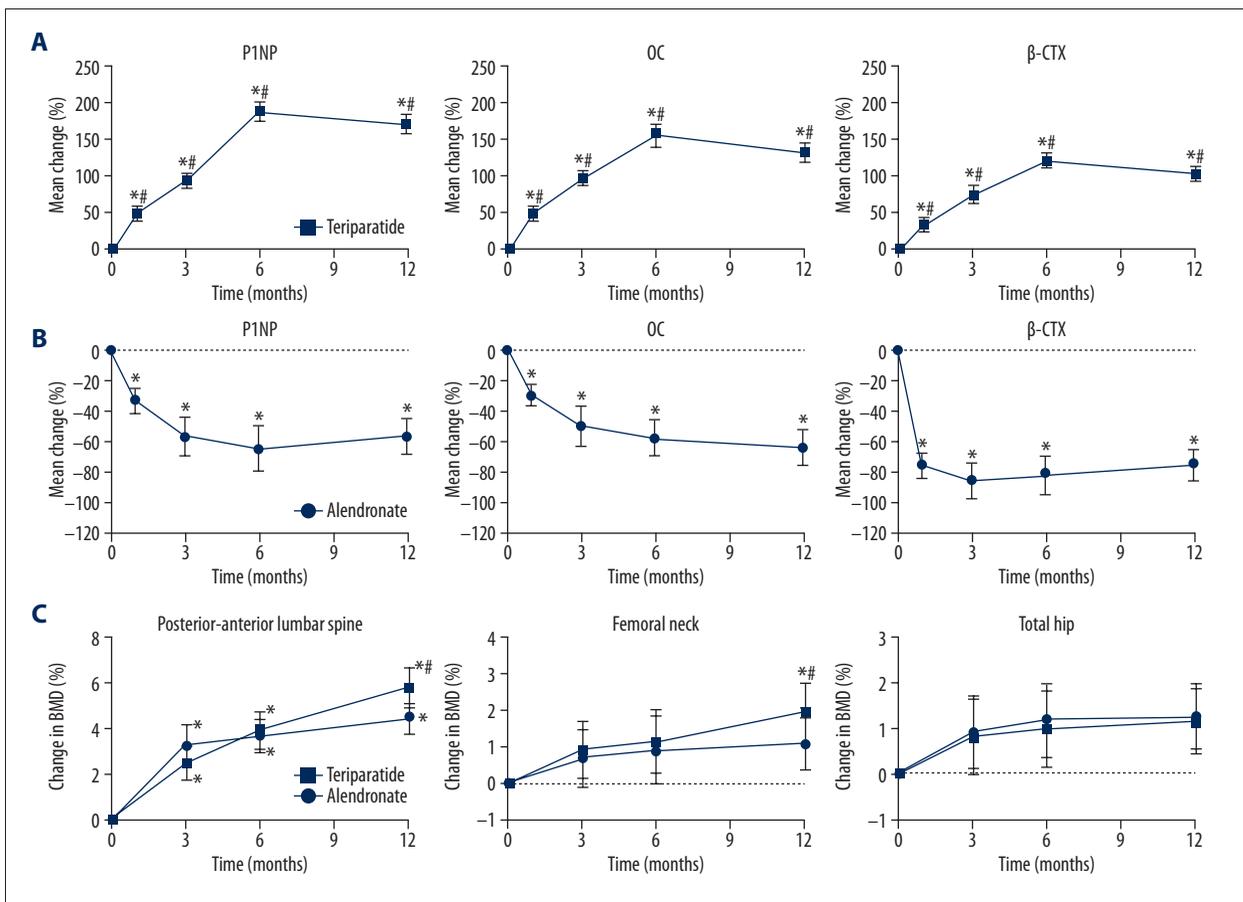

Figure 2. Mean percent changes in bone-turnover markers (P1NP, OC, and β-CTX) from baseline to 12 months in teriparatide (**A**) and alendronate (**B**) groups and mean percentage changes in bone-mineral density (BMD) in 2 groups (**C**). * *P*<0.05 versus baseline, # *P*<0.05 versus alendronate. Data presented are mean ± standard deviation.
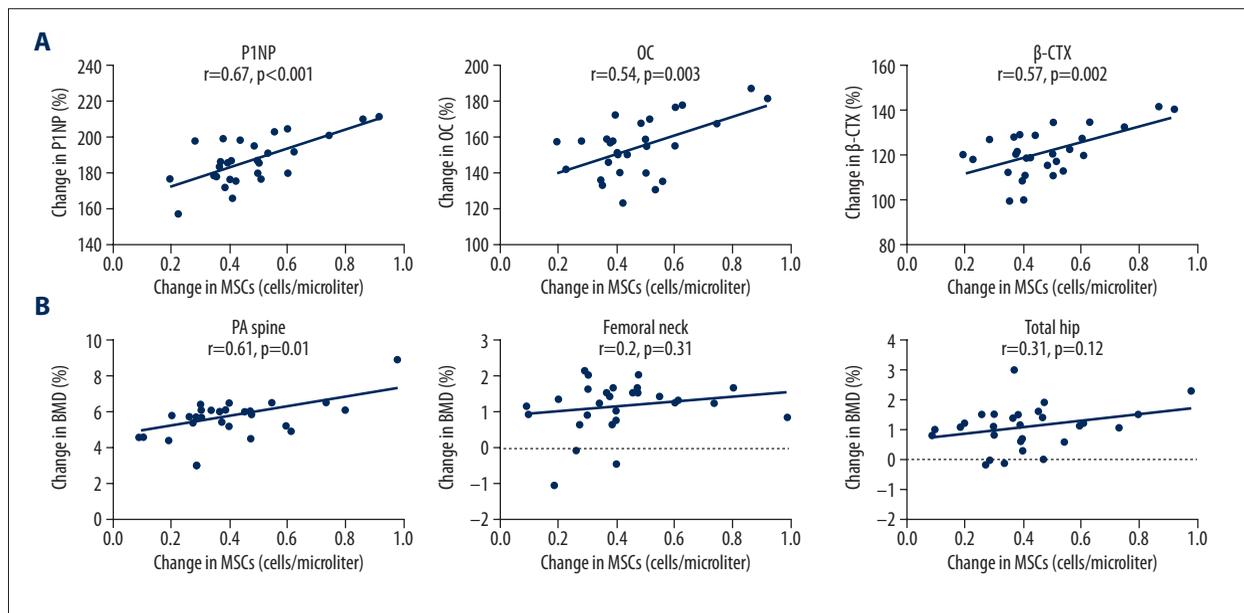
Figure 3. Correlation of change in mesenchymal stem cells (MSCs) with change in bone-turnover markers (**A**) and change in bone mineral density (BMD) (**B**). At month 6, the change in MSCs was positively correlated with change in P1NP, OC, and β-CTX. At month 12, the change in MSCs was positively correlated with change in posterior-anterior lumbar (PA) spine BMD, but was not associated with change in femoral neck or total hip BMD.

compared to baseline. There was no significant change in the number of MSCs in the alendronate treatment group at month 1 (P=0.302), month 3 (P=0.051), month 6 (P=0.514), and month 12 (P=0.053) compared to baseline. Circulating MSCs were significantly more numerous in the teriparatide group than in the alendronate group at all-time points (P<0.001).

Changes of serum biochemical markers and BMD were positively associated with changes of circulating MSCs

Mean (±SD) percentage changes in serum P1NP, OC, and β-CTX expression levels during the 12 months are shown in Figure 2. In teriparatide-treated women, mean serum P1NP, OC, and β-CTX increased significantly at all time-points, whereas they decreased at all time-points in alendronate group (P<0.001 for all within-group and between group comparisons).

Mean (±SD) percentage changes in BMD throughout the study period are shown in Figure 2. Mean posterior-anterior spine BMD increased significantly at all time-points compared with baseline in both groups (P<0.01). PA spine BMD was similar between the 2 groups at month 3 (P=0.41) and month 6 (P=0.32) and was significantly higher in the teriparatide group than in the alendronate group at month 12 (P=0.02). Mean percent changes of femoral-neck BMD was significantly higher in the teriparatide group than alendronate group at month 12 (P=0.04), but were similar at month 3 (P=0.21) and month 6 (P=0.15). We found no significant differences in changes of mean total-hip BMD at all time-points within and between the 2 groups.

Pearson's correlation analysis was performed to analyze the correlations of changes in circulating MSCs with changes in bone-turnover markers and BMD in teriparatide group. The increase in MSCs was positively associated with changes in P1NP (r=0.67, P<0.001), OC (r=0.54, P=0.003), and β-CTX (r=0.57, P=0.002) at month 6 (Figure 3A). Similarly, change in MSCs was positively associated with the change in PA-spine BMD at month 12 (r=0.61, P=0.01), but not the femoral neck (r=0.2, P=0.31) or total hip (r=0.31, P=0.12) BMD (Figure 3B).

Characterization and immunophenotype of isolated circulating MSCs

Freshly cultured circulating MSCs displayed gradual growth and a small amount of sparse adherent cells displayed a change in morphology from an elongated shape to a spindle shape after the initial 3~4 days. By day 5 to 7, the circulating MSCs grew more rapidly and displayed typical fibroblast-like or long fusiform morphology. By days 10 to 15, cultured cells approached approximately 80–90% confluence of the adherent population for subculture. After being subcultured every 3 days, the cells displayed relatively homogeneous morphology (Figure 4A).

Immunophenotypic analyses via flow cytometry analysis indicated that the cells at passage 3 robustly expressed mesenchymal lineage markers CD73 (98.12±6.31%), CD105 (96.54±5.44%), and CD90 (95.26±4.37%), but not hematopoietic lineage markers CD34 (0.12±0.07%) and CD 45 (0.07±0.02%) (Figure 4B–4F).
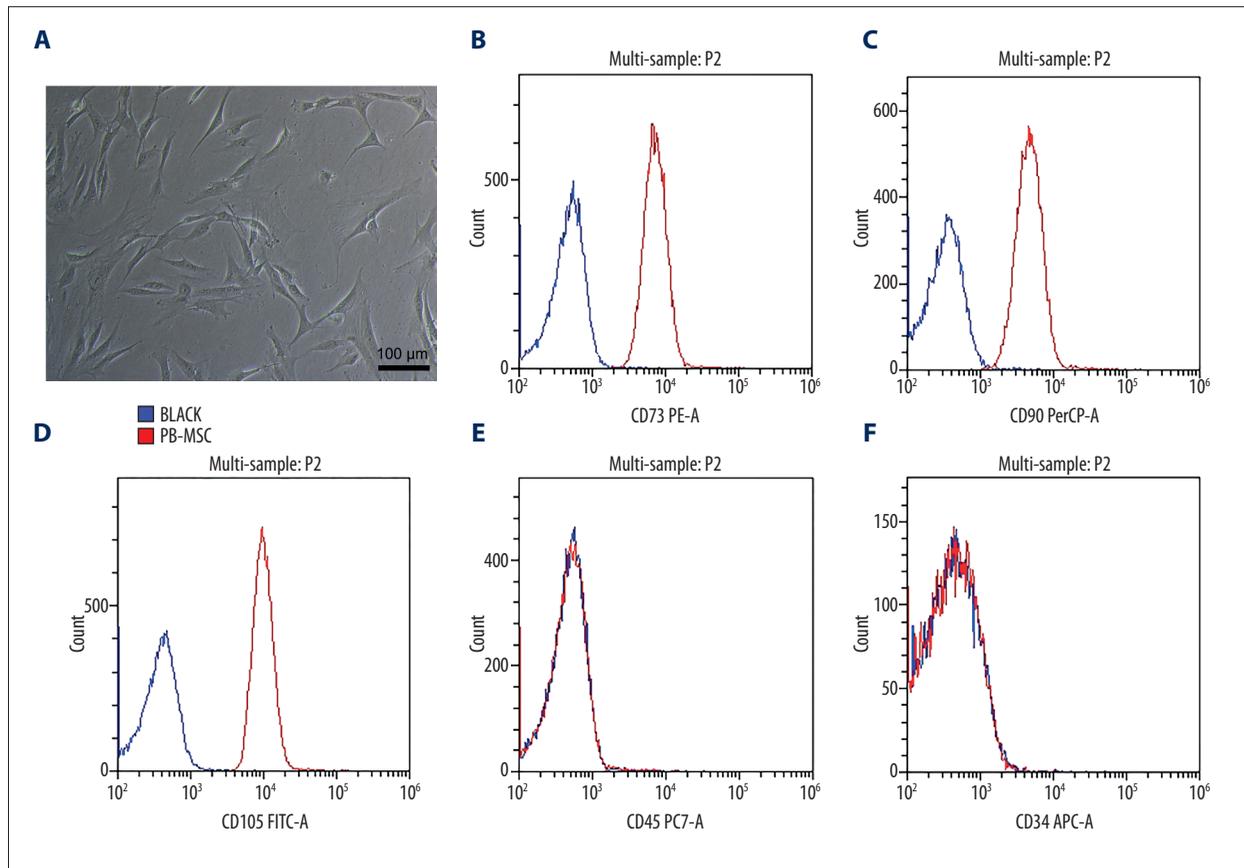
Figure 4. The morphological characteristics of circulating mesenchymal stem cells (MSCs) at passage 3 under an inverted phase contrast microscope (100×) (**A**). Immunophenotypic analyses by flow cytometry indicated passage 3 circulating MSCs were strongly positive for mesenchymal lineage markers CD73, CD90, and CD105 while negative for hematopoietic lineage marker CD45 and CD34 (**B–F**).

Intermittent PTH 1–34 administration enhanced osteogenic differentiation of circulating MSCs

Cultured circulating MSCs underwent osteogenic differentiation upon culturing in osteogenic medium (Figure 5). Compared with the control group, the cells isolated from PTH treated women showed a larger area of CFU-F$_{ALP}$ colonies (Figure 5A) and significantly higher ALP activity (Figure 5C) at day 14 (P=0.004) and day 21 (P<0.001). Similar with ALP staining, the PTH group displayed a larger area of mineralized nodules than the control group upon Alizarin Red S staining (Figure 5B) and a significantly higher calcium deposition volume (Figure 5D) at day 14 (P=0.003) and day 21 (P<0.001). Osteogenesis-associated gene expression was further evaluated between the 2 groups via qRT-PCR analysis. Col 1a1 and OC were steadily upregulated after the first day of osteogenic induction until day 21 (Figure 5E, 5F), transcriptional factors Runx-2 and OSX were gradually upregulated; however, their expression levels peaked at day 14 and then decreased from day 14 to day 21 (Figure 5G, 5H). The genes assessed herein were significantly upregulated in the intermittent PTH 1–34

treated cells than control at all time-points. All these data suggest that PTH 1–34 can enhance osteogenic differentiation of circulating MSCs *in vitro*.

Discussion

In this study, we examined the effect of teriparatide and alendronate administration on circulating MSCs in a human clinical trial. Our results indicated that the number of circulating MSCs of women with postmenopausal osteoporosis significantly increased after 1 month of intermittent PTH 1–34 administration, and this increase persisted till 12 months of treatment. In contrast, no significant changes in circulating MSCs were observed in women administered alendronate treatment. These findings indicate that at least part of the anabolic effects of PTH on bones may be mediated via stimulation of the proliferation of bone marrow MSCs and an increase in circulating MSCs. Furthermore, we report that the increase in circulating MSCs was strongly correlated with PTH increased osteogenic marker expression and spine BMD. These results suggest that
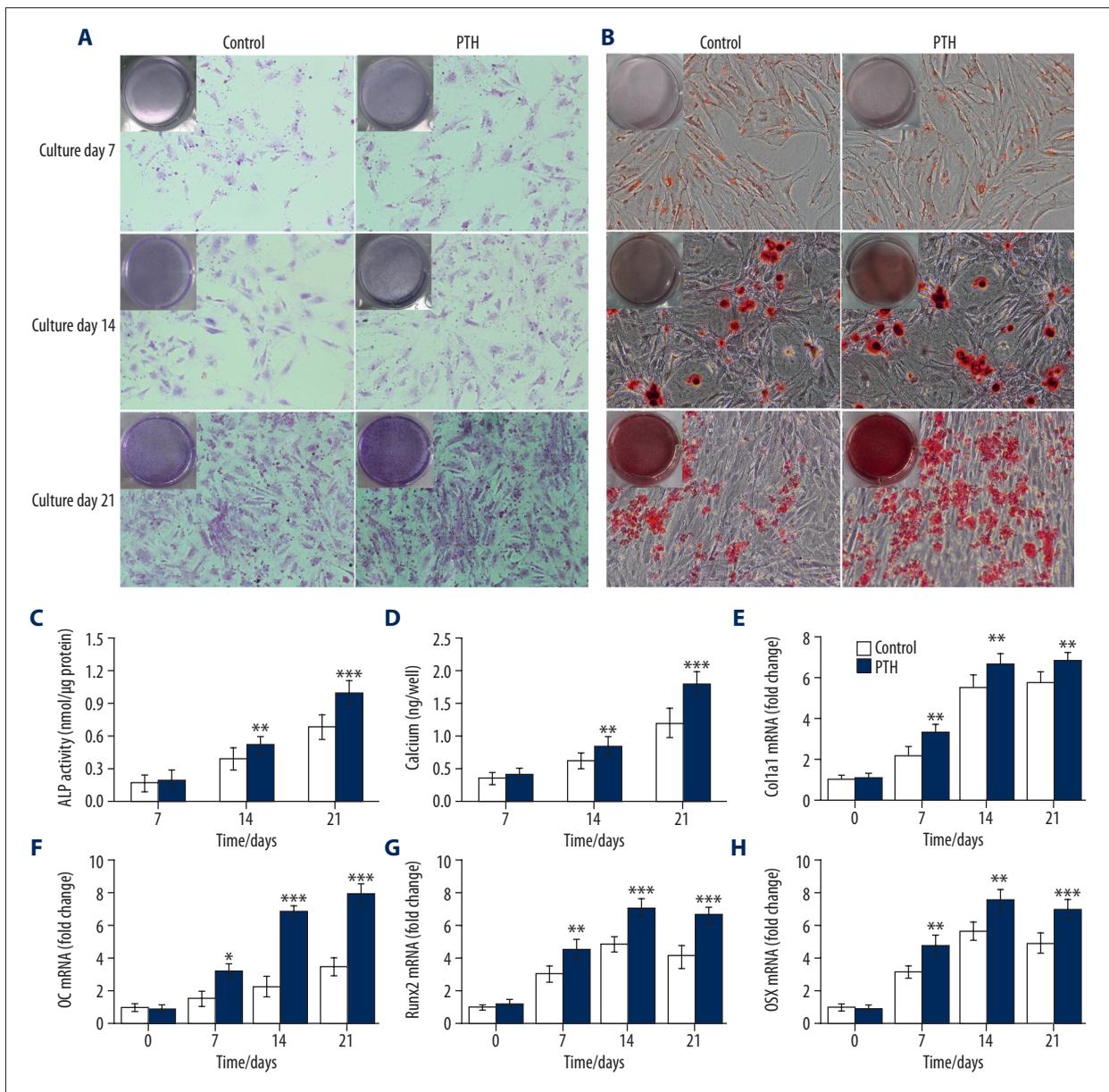
Figure 5. Compared between parathyroid hormone (PTH) and control groups on osteogenic differentiation *in vitro*: histochemical staining for alkaline phosphatase (ALP) (**A**) and mineralized nodule formation was stained by Alizarin Red staining (**B**). The ALP activity and calcium sediment yield were detected by ALP kit (**C**) and calcium kit (**D**). Time course changes in mRNA expression of COL-1a1 (**E**), OC (**F**), Runx-2 (**G**), and OSX (**H**) in mesenchymal stem cells (MSCs) during 21 days of osteogenic induction cultured. * $P<0.05$ as compared to control. ** $P<0.005$ as compared to control. *** $P<0.001$ as compared to control.

increased circulating MSCs may contribute to osteogenesis and increase BMD. Unexpectedly, we failed to establish a clear positive association between changes in MSCs and changes in femur and hip BMD, probably because of the small sample size and the short course of PTH treatment, such that the increases in femur and hip BMD were not evident. More prospective, randomized, interventional, multicenter, long-term clinical trials are required to address this question.

In the *in vitro* analysis, we successfully isolated circulating MSCs from peripheral blood of women with osteoporosis, via Ficoll-based density-gradient centrifugation and elimination of non-adherent cells during culturing. Circulating MSCs are generally believed to be primarily released from the bone marrow. Generally, it may be difficult to isolate these cells from peripheral blood because the amount of circulating MSCs is too small to form colonies under normal conditions [24]. However, circulating MSCs are increased in osteoporosis

patients, thereby enabling the isolation of these cells from peripheral blood [25]. Some studies report the isolation of circulating MSCs upon treatment with mobilizing agents including G-CSF and AMD3100, which are used clinically to mobilize hematopoietic stem cells (HSCs) [26–28]. In the present study, PTH mobilized MSCs to the peripheral blood. This is consistent with the results of some previous animal studies. Sheyn et al. reported that PTH induced systemically administered MSCs to migrate to vertebral and rib fractures in rat models of osteoporosis and multiple-rib-fractures via activation of the SDF1/CXCR4 and amphiregulin/EGFR axes [18,19]. Similar to the effect of PTH on exogenous MSCs, the present results suggest that this agent also influences the recruitment of endogenous bone marrow MSCs. Therefore, the present data provide novel insights into the potential applications of PTH in stem cell therapy for osteoporosis and bone fracture. Moreover, MSCs are not the only targeting stem cells altered in peripheral blood under the effect of PTH. Yu et al. reported that intermittent PTH 1–34 administration can lead to a transient increase of peripheral HSCs in women with postmenopausal osteoporosis [29], while Rubin et al. reported that PTH increases the number of circulating osteogenic cells in hypoparathyroidism [30]. These data suggest that PTH can mobilize and recruit several types of multi-potential stem or progenitor cells from the bone marrow into circulating blood. These cells contribute to regeneration of various injured tissues and organs.

Notwithstanding the increase in circulating MSCs in osteoporosis patients, these cells undergo abnormal osteogenic differentiation [25]. In the present study, intermittent PTH 1–34 administration enhanced the osteogenic differentiation potential of MSCs impaired in osteoporosis, concurrent with previous reports regarding the effects of PTH on bone marrow-derived MSCs (BM-MSCs). Yu et al. reported that PTH stimulates the differentiation of BM-MSCs to the osteoblast lineage by enhancing BMP signaling [17], while Kuo et al. reported that intermittent PTH (1–34) administration enhanced osteogenesis in human BM-MSCs via regulation of protein kinase Cδ [31]. However, the precise mechanisms underlying the enhancement of differentiation of circulating MSCs to the osteoblast

lineage by intermittent PTH administration remains unknown. Considering similar characteristics shared between these 2 cell types [32,33], we have enough evidences to speculate that the mechanisms underlying PTH-induced osteogenesis of circulating MSCs may be consistent with its action on BM-MSCs. Further studies on the molecular mechanism underlying the effects of PTH on circulating MSCs will help address this issue.

This study has several limitations. Although our results provided evidences for enhancement of early MSCs mobilization under daily PTH administration, we could not determine whether this effect was attributed to enhanced egression of BM-MSCs into the circulation or expansion of peripheral MSCs. It was unclear whether these increased MSCs only originate from bone marrow or partly from other different tissues. Unfortunately, we could not test this hypothesis without access to the bone marrow space. Future studies should examine clinical bone marrow specimens in long-term studies with PTH treatment to confirm the preservation and/or expansion of MSCs within the marrow. Our knowledge on circulating MSCs is still limited. Their origin and destination are unclear. Those issues may be resolved in future studies.

Conclusions

Our findings show that intermittent PTH 1–34 administration increased circulating MSCs in postmenopausal osteoporotic women and enhances osteogenic differentiation of these cells *in vitro*. This finding indicates an important role for PTH in MSCs recruitment and illustrates a potential mechanism underlying the anabolic role of PTH on bone formation. Moreover, the present study also provides more evidence regarding the possible application of PTH on tissue regeneration and stem cell transplantation therapy.

Conflict of interest

None.

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