INTRODUCTION

To enhance the fracture healing, a variety of treatment techniques have been developed. Parathyroid hormone peptide 1-34 (PTH 1-34) has been approved by the US Food and Drug Administration for the treatment of post menopausal women with osteoporosis. There is growing evidence that intermittent or continuous treatment with PTH 1-34 accelerate fracture healing [1].

Hematoma occurring at a fracture site is known to play an important role in fracture healing. Recently, we demonstrated that human fracture hematoma-derived cells (HCs) contained progenitor cells with osteogenic/chondrogenic differentiation potential, indicating their critical role in the process of fracture healing [2]. To date, the direct influence of PTH on cells engaging in the process of fracture healing has not been examined. We therefore investigated the direct effect of PTH 1-34 on osteogenic and chondrogenic differentiation of human fracture HCs by intermittent or continuous PTH 1-34 treatments in vitro.

METHOD

Patient characteristics: This study was approved by the institutional ethical committee and informed consent was obtained from all study subjects. Fracture hematoma was obtained from 7 patients.

Isolation and culture of human fracture HCs: Hematoma was harvested from the fracture site and cultured in the growth medium [3].

PTH treatments: HCs were divided into four groups for osteogenic differentiation assay: Control (growth medium), PTH (-) (osteogenic medium (OM) without PTH treatment), PTH-C (OM with continuous PTH treatment), and PTH-P (OM with pulsatile PTH). The OM consisted of β-glycerophosphate and ascorbic acid. For PTH-P group, cells were exposed to 100 nM PTH for the first 6 hour of each 48 hour incubation cycle. In PTH-C group, cells were continuously exposed to 100 nM PTH. For chondrogenic differentiation, pellet culture was performed. The pellets were divided into two groups: PTH (chondrogenic medium (CM) without PTH treatment) and PTH-C (CM with continuous PTH treatment), and cultured for 3 weeks. CM consisted of DMEM with dexamethasone, ITS+, TGF-β3, and BMP-6.

Cell Proliferation Assay: MTS assay was performed.

Alkaline phosphatase (ALP) activity assay: ALP activities of extracted samples were assayed by measuring the release of p-nitrophenol from p-nitrophenyl phosphate as substrate.

Real-time Polymerase Chain Reaction (PCR) Analysis: Expression of osteoblast-related genes, Runx2 and ALP was measured by real-time PCR. For chondrogenesis-related genes, Collagen-II and -X mRNA were measured. The level of each target gene was expressed relative to the day-0 culture levels (ΔΔΔ).

Histological Analysis: The pellets were stained with Safranin-O.

Statistical Analysis: Wilcoxon T tests was conducted to determine whether statistical significance existed at P value (*, P < 0.05).

RESULTS

Cell Proliferation Kinetics: There was no significant difference in the proliferation kinetics of HCs among the four groups (Fig. 1).

ALP activity: At day 14, ALP activity in PTH (-) group and PTH-C group was significantly higher than in the other two groups (Fig. 2).

Gene expression of osteoblast-related genes: The gene expression of Runx2 (Fig. 3A), ALP (Fig. 3B), and OC (Fig. 3C) in PTH (-) group and PTH-C group was significantly higher than in the other two groups at day 14.

Mineralization: HCs in PTH (-) and PTH-C was strongly stained with Alizarin red S at day 14, whereas pulsatile treatment of PTH resulted in weak staining (Fig. 4).

Mineralization Assay: The size of pellet cultured with PTH was smaller than that of pellet cultured without PTH. Pellet without PTH treatment resulted in a greater extent of proteoglycan deposition (Fig. 5).

DISCUSSION

This is the first study that demonstrated the effects of continuous and pulsatile PTH 1-34 treatments on in vitro proliferation and osteogenic/chondrogenic differentiation of human fracture HCs. PTH treatment did not affect cell proliferation. Osteogenic differentiation was not significantly affected with continuous PTH treatment compared to OM without PTH treatment, and found to decrease with pulsatile PTH treatment. Chondrogenic differentiation was inhibited by continuous PTH treatment.

Previously, pulsatile PTH treatment on osteoblasts was reported to promote osteogenic differentiation, whereas continuous PTH treatment inhibited it [3]. On the contrary to previous studies, our study using HCs revealed that pulsatile PTH treatment inhibited osteogenic differentiation, whereas continuous PTH treatment did not inhibit osteogenesis and inhibited chondrogenesis. Our results indicated that effect of treatment with PTH, either continuous or pulsatile, on HCs may act differently compared to other types of cells such as osteoblasts and BMSCs.

SIGNIFICANCE

This study may provide new evidence for the clinical application of local and systemic application of PTH 1-34 for enhancement of fracture healing.

REFERENCES: