Effect of Low-Intensity Pulsed Ultrasound on BMP7-induced Osteogenic Differentiation of Human Nonunion Tissue-Derived Cells in Vitro

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INTRODUCTION

Although fracture healing often occurs without complications, a significant proportion (5 to 10 %) of fractures fail to heal and result in delayed union or persistent nonunion [1]. To enhance the fracture healing, a variety of treatment techniques have been developed. Bone morphogenetic protein-7 (BMP-7) has been reported to promote bone formation in nonunions [2]. It is also well-recognized that low-intensity pulsed ultrasound (LIPUS) accelerates healing of nonunions [3]. It is possible that combination of the two therapies may provide better results than with either treatment alone.

Recently, we demonstrated that human nonunion tissue-derived cells (NCs) contained progenitor cells with osteogenic/chondrogenic differentiation potential in vitro [4]. We hypothesized that the combined application of LIPUS and BMP-7 would enhance osteogenesis of NCs. We investigated whether LIPUS promoted BMP-7-induced osteogenic differentiation of NCs in vitro.

MATERIALS and METHODS

Patient characteristics: Nonunion tissue was obtained from 7 patients undergoing surgical treatment for their nonunions.

Isolation and culture of NCs: NCs were isolated from nonunion tissue, and were cultured in the growth medium. 1 x 10^6 cells per well were seeded onto a 6-well plate. After 4 days, the cells were divided into four groups: (1) BMP-7 group: NCs cultured in osteogenic medium (OM) without ultrasound stimulation, and (2) BMP-7 + LIPUS group: NCs cultured in OM with LIPUS treatment. The OM consisted of the growth medium supplemented with 10 mM β-glycerophosphate, 50 μg/ml ascorbic acid, and 1 ng/ml recombinant human BMP-7.

Ultrasound stimulation: We used a LIPUS exposure device. This device produces a wave equal to the wave conditions of sonic explosion occurring in nonunions [2]. It is also well-recognized that low-intensity pulsed ultrasound (LIPUS) accelerates healing of nonunions [3]. It is possible that combination of the two therapies may provide better results than with either treatment alone.

Recently, we demonstrated that human nonunion tissue-derived cells (NCs) contained progenitor cells with osteogenic/chondrogenic differentiation potential in vitro [4]. We hypothesized that the combination of LIPUS and BMP-7 would enhance osteogenesis of NCs. We investigated whether LIPUS promoted BMP-7-induced osteogenic differentiation of NCs in vitro.

Ultrasound stimulation: We used a LIPUS exposure device. This device produces a wave equal to the wave conditions of sonic accelerated fracture healing system for clinical use. LIPUS was given through the bottom of the culture plates for 20 minutes daily at 37°C [5].

Cell proliferation assay: The concentration of DNA in each sample was determined using PicoGreen assay. The concentration of DNA in each sample was expressed relative to the day 0 culture levels.

Alkaline phosphatase (ALP) activity assay: ALP activities of extracted samples at days 7 and 14 were assayed by measuring the release of p-nitrophenyl phosphate from p-nitrophenyl phosphate as substrate.

Real-time Polymerase Chain Reaction (PCR) Analysis: Expression of osteoblast-related genes, ALP, runt-related gene 2 (Runx2), osterix (OSX), osteocalcin (OC), and GAPDH was measured by real-time PCR. The level of each target gene was normalized to GAPDH levels and expressed relative to the day 0 culture levels. The level of OSX, Runx2, and OC mRNA expression was found between BMP-7 alone group and BMP-7 + LIPUS group (Fig. 3).

DISCUSSION

Our results clearly showed for the first time that LIPUS promoted BMP-7-induced osteogenesis of NCs by up-regulation of ALP activity, gene expression of ALP and Runx2, and the higher calcium deposition. There was no significant effect of LIPUS treatment on the proliferation of NCs, indicating LIPUS does not affect cell proliferation. The up-regulation of OC and osterix was not seen within 14 days. Future studies will include the verification of transition of these markers at later time points.

The therapeutic potential of application of BMP-7 and LIPUS for the enhancement of nonunion treatment has been well documented in clinical reports [6, 7]. Our study indicated that the combination of local application of BMP-7 and LIPUS treatment at the nonunion site may enhance the healing of nonunion by enhancing osteogenic activity of NCs within nonunion tissue.

SIGNIFICANCE

Our findings suggest that the combination of local application of BMP-7 and LIPUS treatment at the nonunion site may become a new nonunion therapy alternative to the conventional invasive treatment such as autogenous bone grafting.