Intermittent administration of Human Parathyroid Hormone (1-34) Accelerates the Maturity of Spinal Fusion Mass in Rat Model.

INTRODUCTION: Bone graft is a commonly used procedure to treat skeletal disorders such as tumor, trauma, pseudoarthrosis and degenerative spinal diseases. Of those disorders, spinal fusion is the most frequently used application of bone graft and the efficacy of this surgical procedure is widely accepted. [1] However, it usually takes several months to achieve solid union after spinal fusion surgery and the patients are required to restrict the activity of daily living during this period.

Intermittent administration of parathyroid hormone (PTH) has been demonstrated to have potent anabolic effects on bone remodeling. Currently, it has been shown not only to prevent loss of bone mass in patients with osteoporosis, but to accelerate the fracture healing in experimental animal models. [2, 3] Therefore, we hypothesized that intermittent administration of human PTH (1-34) shortens the period of graft bone healing in spinal fusion surgery.

The objective of this study is to clarify the effect of intermittent administration of human PTH (1-34) on graft bone healing in rat posterolateral spinal fusion (PLF) model.

METHODS: Eighty-two male Sprague-Dawley rats (eight weeks of age) were used. All rats underwent posterolateral spinal fusion at L4-5 using autogenous iliac bone graft. Animals were divided into two groups and given subcutaneous injections of hPTH (1-34) (40 µg/kg/day) (PTH group) or 0.9% saline vehicle (control group) respectively.

Experimental study 1
Animals were given injection 7times a week. Five rats of each group were euthanized at two, four, seven, and 14 days after surgery and grafted bone was harvested. Time course expression of bone related genes in grafted bone:

Time course expression of bone related genes in grafted bone: The expression level of mRNA for bone related genes (insulin like growth factor I (IGF-I), alkaline phosphatase (ALP), osteocalcin (OC), osteonectin (ON), type I collagen (COL1A1), type II collagen (COL2A1), type X collagen (COL10A1), calcitonin receptor (CTR), tartrate-resistant acid phosphatase (TRAP)) were analyzed by real-time reverse transcription- polymerase chain reaction (RT-PCR).

Experimental study 2
Animals were given injection Stimes a week. At 14, 28 and 42 days after surgery, seven rats of each group were euthanized and the lumbar spine was harvested. Rats were double-labeled with a subcutaneous injection of calcine (10 mg/kg) at 9 days and 2 days before death. Fusion assessment: Solid fusion was defined as no motion on transverse processes on soft X-ray images.

Microstructural analysis of fusion mass:

Microstructural analysis of fusion mass: The values of trabecular thickness (Tb.Th) and cortical thickness (Ct) increased during first 28 days, and afterwards decreased with the maturation of fusion mass in control group, while these values reached a peak on day 14 and decreased from day 14 to day 42 (Fig. 2A and B). The values of trabecular thickness (Tb.Th) and cortical thickness (Ct) increased in both control and PTH group, but PTH group on day 28 obtained same values of Tb.Th and Ct in controls on day 42 (Fig. 2C and D).

RESULTS:

Microstructural indices analysis was performed using reconstructed 3D-CT images. Histomorphometry: The undecalified specimens sectioned to a thickness of 3µm in axial plane were prepared and observed by fluorescent microscopy. Mineral apposition rate (MAR) was measured. Serum analysis: Serum osteocalcin (OC) and type I collagen cross linked C-telopeptides (CTX) levels were assessed using an ELISA.

DISCUSSION: The results of the present study indicate that intermittent administration of hPTH (1-34) accelerates the graft bone healing resulting in shortening the period of maturation of fusion mass in rat spinal fusion model. The mechanism for this is supposed that intermittent administration of hPTH enhanced both bone formation and bone resorption in grafted bone. Higher expression of both osteoblast and osteoclast related genes and higher mineral apposition rate in PTH group support this idea. Also, higher value of serum bone metabolic markers in PTH group reflects the higher bone turn over status in grafted bone. Actually, PTH treated animals achieved structurally matured fusion mass till 28 days after surgery which is equivalent to that at 42 days after surgery in control group in both control and PTH group.

In conclusion, intermittent administration of hPTH (1-34) could become an efficient adjuvant intervention of spinal fusion surgery and all other orthopaedic surgery required bone grafting.

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Fig. 1. Micro-CT image of fusion mass

Fig. 2. Microstructural indices analysis of fusion mass.

Fig. 3. Bone metabolic markers.

Microstructural analysis showed that BV/TV and N.Nd/TV increased during first 28 days, and afterwards decreased with the maturation of fusion mass in control group, while these values reached a peak on day 14 and decreased from day 14 to day 42 (Fig. 2A and B). The values of trabecular thickness (Tb.Th) and cortical thickness (Ct) increased in both control and PTH group, but PTH group on day 28 obtained same values of Tb.Th and Ct in controls on day 42 (Fig. 2C and D).

Microstructural analysis of fusion mass: Micro CT showed that grafted bone was resorbed and remodeled to solid fusion mass during first 28 days after surgery in PTH group, while fusion mass was immature and grafted bone fragments still remained on day 28 in the control group (Fig. 1).

Day 14 Day 28 Day 42

CNT PTH

A B C

D E F

Serum analysis: Both serum OC and CTX levels were higher in PTH group compared to those in control group through observation period (Fig. 3).

Histomorphometry: MAR was significantly higher in PTH group both on day 28 (3.15µm/day in controls; 4.31µm/day in PTH: p<0.001) and on day 42 (2.25µm/day in controls; 4.05µm/day in PTH: p<0.001) than in control group.