The Effect Of Parathyroid Hormone(1-34) On Articular Cartilage Surface Lesion Induced Post-traumatic Osteoarthritis

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Introduction: Articular cartilage is lack of self-repair ability. Articular injuries, caused by cartilage impaction, ligament injury and surface incongruity, may induce post-traumatic osteoarthritis (PTOA). Unfortunately, there are no effective clinical treatments to suppress the progression of PTOA. Previous reports suggested that early interventions might halt PTOA progression by suppressing chondrocyte apoptosis, produced pro-inflammatory cytokines, and matrix-degrading enzymes. In previous study, we found parathyroid hormone(1-34), PTH(1-34), reversed type 2 collagen and glycosaminoglycan loss, and decrease chondrocyte apoptosis in papain- and anterior cruciate ligament transaction-induced OA rats. However, the effect of PTH(1-34) in articular cartilage surface lesion-induced PTOA has not been evaluated. In this study, we hypothesize that early intervention of PTH(1-34) in cartilage surface lesion may suppress chondrocyte death and cartilage matrix loss, and thus suppress PTOA progression.

Methods: Fifty-six 12-week-old SD rats were used in this study to create the partial thickness chondral lesions at patellar groove in 212 femur heads and 6 of them as the non-lesion intact control. Two traversed lesions were made and the interval between two lesions was around 3mm, and depth of lesion was 300 um. There were two groups in this study: the left joints of the rats were injured as the lesion group, and the right joints were injured and treated with PTH(1-34) as the treatment group. In the treatment group, 40 ul of 10 nM PTH(1-34) was administrated by intra-articular injection every 3 days. We evaluated cell apoptosis and glycosaminoglycan (GAG) change at immediate after surgery, and day 1 and day 3 after surgery (acute phase). The changes of morphology and were evaluated GAG at day 14 and 35 after surgery (subsequent phase). The cell apoptosis was detected by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining. The GAG change was evaluated by safranin O-fast green staining. The quantitation of GAG staining intensity and cell apoptotic rate were analyzed by Image Pro Plus software.

Results: The success rate for creating partial thickness chondral lesion was 72.6%. The failure for creating lesion includes no lesions, damage to subchondral bone, and inflammation. After histological evaluation, we found that GAG loss in surrounding tissue of lesions at acute stage of injury, and no self-repair of GAG content was found till 14 days after injury in the lesion group. Obvious chondrocyte apoptosis was found in the surrounding tissue of lesion site at 1 day after injury and apoptotic cells spread into tidemark at 3 days after injury. Comparing with the lesion group, apoptotic rate was 30% decreased in the PTH(1-34) treatment group. For chondrocytic death, less chondrocyte apoptosis and loss were found in the treatment group comparing with the lesion group. For cartilage matrix loss, PTH(1-34) treatment reduced GAG loss in the treatment group rather than in lesion group using quantitative analysis. The future work is to detect other matrix molecules, such as Col 1, and Col 2, for ensuring the effect of PTH(1-34) on suppressing PTOA progression.

Discussion: 1. Partial-thickness cartilage lesions leaded to cell death, cartilage matrix loss, and articular cartilage structure degeneration during development of PTOA progression. 2. Repair processes are correlation about direction of injuries. Longitudinal Injury repair process is better than transverse injury. And, the effect of PTH(1-34) on suppressing PTOA progression needs more study to confirmed.

Significance: We established a method of scratch articular surface lesion that led GAG loss and chondrocyte apoptosis in the surrounding tissue of the lesion site 1 day after injury, and the gradual GAG loss was be observed till 5 weeks after injury. PTH(1-34) treatment reduced injury-induced chondrocyte apoptosis, thus prevent cell loss in lesion area.

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Lesion group
Lesion-induced PTOA without PTH(1–34) treatment

12 weeks-old SD RAT

Treatment group
10 nM PTH(1–34) treatment (every 3 days by intra-articular injection) followed by lesion-induced PTOA

Surgical day
D0

D1
D3
D7
D14
D35

Measure cell apoptosis and extracellular matrix change

Morphology and molecular changes in ECM

Extracellular matrix

<table>
<thead>
<tr>
<th>Examination</th>
<th>Method</th>
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<tbody>
<tr>
<td>Cartilage lesion</td>
<td>Gross examination-Indian ink</td>
</tr>
<tr>
<td>Glycosaminoglycan</td>
<td>Safranin O-fast green staining</td>
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<tr>
<td>Cell loss</td>
<td>DAPI staining</td>
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<tr>
<td>Cell apoptosis</td>
<td>TUNEL staining</td>
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</table>
The 2 transverse lesions of Depth are 300um on patellar groove of femur.

Did Longitudinal direction wax section

Indian ink stain

TUNEL

Merge

DAPI

Lesion

Ctrl