SKELETAL RELOADING EFFECTS RECOVERY OF THICKNESS OF THINNED CARTILAGE BUT NOT CARTILAGE DEFECT INDUCED BY UNLOADING WITH AN INCREASE OF SERUM TYPE II COLLAGEN SYNTHESIS MARKER

Introduction:
Previously we provided evidence that skeletal unloading by tail suspension induces a full-thickness patellar cartilage defect at the far medial margin and an increase in the urinary collagen II CTx degradation marker in growing rats (1). Also our results in relation to the lateral part of the patellar cartilage was coincided with those on the effect of skeletal unloading on tibiofemoral articular cartilage (2). However, little is known of the effects of skeletal reloading on articular cartilage and subchondral bone after unloading, that is, whether the pathogenesis of articular cartilage defects was induced by unloading is reversible. Thus, we investigated the effects of skeletal reloading on articular cartilage and bone after unloading using the tail suspension model in growing rats.

Materials and Methods:
Thirty-six 9-week-old male F344/N rats were randomly divided into three groups: caged control (C), tail-suspended (TS), and reloaded (TS+RL). Hindlimbs of the TS rats were subjected to unloading for 3 weeks by the Morey-Helot method (3, 4) with some modification. The TS+RL rats were subdivided into two groups. After unloading for 3 weeks, hindlimbs of the TS+RL rats were allowed to reload for 3 or 6 weeks (TS+RL3w, TS+RL6w). All animals were injected subcutaneously with calcine (20 mg/kg) at 7 or 9 and 2 days before sacrifice to label the tidemark and the mineralizing bone surface. Decalcified sections and undecalcified sections were made from paraffin-embedded patellas and methylmetacrylate-embedded patellas, respectively. The sequential changes of patellar cartilage and bone were analysed by pathological findings using hematoxylin–eosin stain and safranin-O stain. Cartilage defect ratio (CDR), total cartilage area (TCA), calcified cartilage area (CCA)/TCA, mean cartilage thickness (MCT), number of vascular invasions from the subchondral bone to the cartilage layer and the tidemark mineral apposition rate (tidemark MAR) both in the medial and lateral facets were analysed in the section at the level of the distal one-third of the patella by personal computer using NIH image 1.62. The values of type II procollagen-C-peptide (pColII-C) which is a useful index of type II collagen synthesis (5) in sera obtained at sacrifice were also measured by EIA. The study protocol was approved by the Guiding Committee of the Center for Laboratory Animal Science, National Defense Medical College.

Results:
Pathological findings demonstrated that in the TS group a full-thickness patellar cartilage defect was found at the far medial margin of the patella without any fibrillation at the remaining articular surface and that the patellar cartilage thickness was decreased in the medial but not in the lateral part of the patella. Safranin-O staining was reduced in the interterritorial matrix especially at the medial part. In the TS+RL groups, the full thickness cartilage defect remained but cartilage thickness recovered in the medial part compared with the findings for the C group without any fibrillation at the remaining articular surface. Also safranin-O staining had recovered without a difference between the medial and lateral parts.

Image analysis demonstrated that in the TS group, the CDR significantly increased and the TCA and MCT in the medial part significantly decreased compared with the C group. In the TS+RL groups, the CDR significantly increased and the TCA in the medial part significantly decreased compared with the C group. However, the MCT in the medial part did not differ significantly from that in the C group (Fig.1). In the TS group, the CCA/TCA and tidemark MAR in the lateral part, where a cartilage defect was not found, increased significantly compared with the C group. In the TS+RL3w group, those values significantly decreased, but did not differ significantly in the TS+RL6w group from those in the C group (Fig.2). The number of vascular invasions from the subchondral bone to the cartilage layer in the TS group tended to be decreased, but in the TS+RL3w, was significantly increased compared with the C group. In the TS group, the serum pColII-C level was significantly decreased compared with the C group, but in the TS+RL3w group this value was significantly elevated compared with the C group (Fig.3).

Discussion:
In this study, skeletal reloading resulted in recovery of the thinned cartilage and loss of safranin-O staining induced by skeletal unloading without any fibrillation at the remaining articular surface. However, with skeletal unloading, the decrease in the TCA did not reverse with unloading. These results indicated that skeletal reloading could effect recovery of thinned articular cartilage but could not reverse a full-thickness cartilage defect. That is, once such a defect was induced by unloading, reloading could not regenerate cartilage tissue at the site. Our results also demonstrated that skeletal unloading significantly decreased type II collagen synthesis and that skeletal reloading significantly increased the synthesis of type II collagen. We have already shown that skeletal unloading increased the urinary collagen II CTx degradation marker (1). Therefore, our results indicated that skeletal unloading induced uncoupling of type II collagen synthesis and degradation, which was consistent with the pathological findings.
Skeletal reloading restored the ratio of the CCA/TCA that had been decreased by skeletal unloading (2). The process of recovery of the ratio of CCA/TCA consisted of an increase of the uncalcified cartilage layer and a decrease of the calcified cartilage layer. Our results suggested that the former may be associated with an increase of type II collagen and proteoglycan synthesis in articular chondrocytes by reloading and that the latter resulted from a significant increase in the number of vascular invasions from the subchondral bone to the cartilage layer, which meant an increase in the replacement of calcified cartilage with bone, and a significant deceleration of the advancement of the tidemark toward the articular surface. Together this study indicated that it was very important to prevent the full-thickness cartilage defect in unloaded condition.

Conclusion:
1. Reloading effect recovery of thickness of the entire thinned cartilage was induced by unloading without any fibrillation at the remaining articular surface.
2. Reloading decelerated the tidemark advancement toward the articular surface and decreased the ratio of CCA/TCA at the early phase and restored these values thereafter.
3. Reloading increased the serum type II collagen synthesis marker.
4. Reloading did not result in recovery of a full-thickness cartilage defect.

References:

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