Follistatin alleviates articular cartilage degradation induced by carrageenan.
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INTRODUCTION:
Activins, which belong to the Transforming Growth Factor-beta/ Bone Morphogenetic Protein (TGFβ/BMP) superfamily, are circulating cytokines involved in the process of inflammation of various tissues [1]. Follistatin is an endogenous extracellular inhibitor for activins which binds and interfere the interaction between activins and their receptors. Jones et al showed that administration of follistatin reduced inflammatory response mediated by activins and mortality in endotoxicemia in mice [2]. These data enhance the importance of activin signals in the pathogenesis of inflammation-mediated diseases and follistatin as a therapeutic target for them.
Carrageenan is a sulphatedmucopolysaccharide derived from the Irish moss Chondrus crispus or from red Scottish seaweed. It is known for its remarkable capability to stimulate local inflammation dominated by intense macrophage aggregation and by fibroblastic proliferation. It is reported that single intra-articular injection of carrageenan initiates a localized synovial inflammatory response, which results in a decrease in both the proteoglycan content and in the rate of proteoglycan synthesis in the articular cartilage [3]. Here we report that follistatin alleviates articular cartilage degradation induced by carrageenan.

METHODS:
This study was approved and conducted in accordance with the guideline of the animal committee of Tokyo Medical and Dental University. Male C57Bl/6J mice (12weeks old) were purchased from ORIENTAL YEAST co., Ltd (Tokyo, Japan). They were housed under a 12-h light-dark cycle and allowed food and water ad libitum. Twelve mice were randomly divided into two groups (n=6/group). Mice were anesthetized by the inhalation of 5% isoflurane in oxygen. Under deep anesthesia, a solution of 30µg lambda-carrageenan (Sigma-Aldrich) in 5µL saline was injected into the left knee joint through the lateral margin of the patella tendon. Recombinant mouse follistatin (25ng in 5µL in physiological saline, Sigma-Aldrich) was injected into the left knee joint at 30 min before carrageenan challenge. The animals were sacrificed at 3 days after the challenge. Knee joints were dissected, fixed in 4% paraformaldehyde, decalcified in 20% EDTA (pH 7.4), and embedded in paraffin. Five µm-thick sagittal sections (medial condyle) were prepared and stained with safranin-O/fast green to evaluate the extent of cartilage proteoglycan loss. To evaluate the loss of proteoglycan from articular cartilage, we developed semi-quantitative scoring system indicated in table 1. Histological scores for femurs and tibiae were evaluated independently and total scores were compared between the two groups. The results were presented as mean +/- SD. Statistical analyses were performed using Mann-Whitney’s U test and p values <0.05 were considered significant.

RESULTS:
As shown previously, dyeability of articular cartilage by safranin-O was significantly reduced by the single intra-articular injection of carrageenan at 3 days (Fig1 upper panel). However, we did not observe obvious alteration in the articular surface structure at this stage. Interestingly, such a loss of dyeability was not observed by the pre-injection of follistatin (Fig1 lower panel). We repeated this experiment six times and observed a similar result in each experiment.

To evaluate the anti-catabolic effect of follistatin semi-quantitatively, we employed a scoring system shown in table 1. As shown in Fig2, pre-injection of follistatin significantly rescued a loss of dyeability induced by carrageenan (p<0.05).

DISCUSSION
Carrageenan-induced arthritis is a well-established experimental model to investigate inflammation-mediated articular cartilage degradation in rodents. Here we report that follistatin effectively inhibits the loss of safranin-O dyeability of articular cartilage induced by carrageenan. Our data strongly suggest that the activin/BMP signal pathway is involved in the process of joint inflammation and proteoglycan loss in the cartilage matrix. Further investigation is required to determine the immunobiological effect of follistatin in inflammatory arthritis.

CONCLUSION:
Follistatin alleviates articular cartilage degradation induced by carrageenan.

SIGNIFICANCE:
Our results support a potential therapeutic application of follistatin for arthritis.

REFERENCES:

Table 1. Histological evaluation of articular cartilage degradation

<table>
<thead>
<tr>
<th>Score</th>
<th>Criteria</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>Loss of safranin-O without structural changes in less than 30% of articular surface</td>
</tr>
<tr>
<td>2</td>
<td>Loss of safranin-O without structural changes in between 30% to 60% of articular surface</td>
</tr>
<tr>
<td>3</td>
<td>Loss of safranin-O without structural changes in more than 60% of articular surface</td>
</tr>
<tr>
<td>4</td>
<td>Loss of safranin-O with structural changes in less than 30% of articular cartilage</td>
</tr>
<tr>
<td>5</td>
<td>Loss of safranin-O with structural changes in between 30% to 60% of articular cartilage</td>
</tr>
<tr>
<td>6</td>
<td>Loss of safranin-O with structural changes in more than 30% of articular cartilage</td>
</tr>
</tbody>
</table>