HYDROSTATIC PRESSURE INDUCES APOPTOSIS OF CHONDROCYTE CULTURED IN ALGINATE BEADS

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Discussion:
Osteoarthritis (OA) is a degenerative joint disease that is characterized by articular cartilage degradation. Recently, a new theory for the onset and development of OA has suggested that chondrocyte apoptosis might be involved. Some factors, such as nitric oxide (NO), retinoic acid and Fas antigen, have been reported to induce programmed cell death of chondrocytes.

Articular cartilage is always subjected to various mechanical stresses. Excessive mechanical stress has long been recognized as a deleterious factor for the cartilage matrix and as one of the major causative factors in OA. However, it still remains unknown that mechanical stresses have any influence on chondrocyte apoptosis. In the present study, we investigated the influence of hydrostatic pressure (HP) on apoptosis of chondrocytes cultured in alginate beads.

Methods:
Chondrocytes were isolated from articular cartilage of rabbit joints and seeded in alginate beads. Beads were divided into two groups. The first group (group A) was exposed immediately to HP of 10 or 50 MPa for 12 or 24 hours using an apparatus that can add pressure under sterile conditions. Chondrocytes in alginate beads of the second group (group B) were cultured for two weeks until abundant extracellular matrix accumulated around the cells, then exposed to HP in the same manner. After HP exposure, alginate beads were fixed in 4% paraformaldehyde followed by staining with Safranin O and histological examination was performed with frozen sections of the specimens. Apoptotic cells were quantified using the terminal deoxynucleotidyl transferase-mediated X-dUTP nick end labeling (TUNEL) method.

Results:
Chondrocytes were surrounded with newly formed matrix after two weeks of culture in alginate beads (Fig. 1-b). After the exposure to 50 MPa of HP for 24 hours, nuclear condensation was observed in chondrocytes of group A (Fig. 1-c). Morphological changes were not observed in chondrocytes of group B.

Apoptotic chondrocytes were not observed in the control cells under atmospheric pressure, whereas apoptosis was observed in group A cells and the number of apoptotic cells increased along with the duration and magnitude of HP increased. The apoptosis rates were 11.0% (12 h) and 20.4% (24 h) after the exposure of 10MPa of HP, which increased to 14.2% (12 h) and 59.8% (24 h) after the exposure to 50MPa of HP. On the other hand, there was no significant population of apoptotic cells observed in group B. The apoptosis rates were 0.9% (12 h) and 1.2% (24 h) after the exposure to 10MPa of HP, 1.9% (12 h) and 2.0% (24 h) after the exposure to 50MPa of HP.

Discussion:
In this study, we applied 3-dimensional alginate beads-culture system for primary isolated chondrocytes. This way of cell culture offers several advantages over monolayer culture; dedifferentiated chondrocytes can redifferentiate, and chondrocytes can produce cartilage-specific extracellular matrix and maintain their phenotype for long time. It is a relevant model for the study of articular chondrocyte.

After two weeks of culture in alginate beads, extracellular matrix was newly formed around the cells. These cells were resistant for physiological or excessively strong HP, while excessively strong HP induced apoptosis in cells after short term culture in alginate beads. These results indicated that excessive mechanical stress could evoke apoptosis when the extracellular matrix surrounding the chondrocytes is insufficient.

These findings suggest that mechanical stress might play an important role in pathogenesis related to chondrocyte apoptosis in OA.

Table 1. Rates of apoptotic cells after exposure to hydrostatic pressure. Values are indicated as mean ± S.D. (Each n=5). Chondrocytes cultured in alginate beads under atmospheric pressure are regarded as controls.

<table>
<thead>
<tr>
<th>Hydrostatic pressure</th>
<th>Group A</th>
<th>Group B</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>10MPa 12h</td>
<td>11.01 ± 6.78</td>
<td>0.93 ± 1.01</td>
<td>&lt; 0.05</td>
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<tr>
<td></td>
<td>20.43 ± 5.00</td>
<td>1.19 ± 0.94</td>
<td>&lt; 0.001</td>
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<tr>
<td>50MPa 12h</td>
<td>14.18 ± 3.02</td>
<td>1.85 ± 0.81</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>59.77 ± 7.18</td>
<td>2.01 ± 0.69</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Control</td>
<td>1.74 ± 1.22</td>
<td>0.00 ± 0.00</td>
<td></td>
</tr>
</tbody>
</table>

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Fig. 1. Light micrographs of chondrocytes cultured in alginate beads. Cells were cultured for less than 1 day (a and e) or for 2 weeks (b and d). (a) (b) control cells were cultured under atmospheric pressure ; (c) (d) chondrocytes were exposed to 50 MPa of hydrostatic pressure for 24 hours. Arrows indicate chondrocytes with condensed nuclei. Bar = 50 μm