Crystals and Osteoarthritis

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Objectives

- Review epidemiologic evidence for an etiologic connection between OA and crystals
- Discuss relationship between crystal formation and OA
- Learn how crystal-induced damage worsens OA
Gout prefers joints affected by osteoarthritis

Gout is associated with knee OA

Howard et al.
Crystal formation in gout

- MSU crystal formation occurs in solution
- Promoted by:
  - Cool temperatures
  - acidic pH
  - decreased oxygen saturation
  - Ca2+
  - Gouty synovial fluid
  - Serum
  - Collagen
  - mechanical trauma
  - ? Cartilage matrix components
Immunoglobulins bind to MSU crystals

Immunoglobulins play a role in gout crystal formation

Gout can deposit in or on cartilage
MSU crystals induce mechanical damage
MSU crystals predict worse cartilage damage

<table>
<thead>
<tr>
<th></th>
<th>Total Sample</th>
<th>%</th>
<th>Crystal tali</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td># donors</td>
<td>4007</td>
<td></td>
<td>187</td>
<td>4.7</td>
</tr>
<tr>
<td># individual tali</td>
<td>7855</td>
<td></td>
<td>344</td>
<td>4.4</td>
</tr>
<tr>
<td>Grade 0</td>
<td>2948</td>
<td>38</td>
<td>27</td>
<td>7.8</td>
</tr>
<tr>
<td>Grade 1</td>
<td>2856</td>
<td>36</td>
<td>129</td>
<td>37.5</td>
</tr>
<tr>
<td>Grade 2</td>
<td>1614</td>
<td>20.5</td>
<td>159</td>
<td>46.3</td>
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<tr>
<td>Grade 3</td>
<td>413</td>
<td>5.2</td>
<td>29</td>
<td>8.4</td>
</tr>
<tr>
<td>Grade 4</td>
<td>24</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>mean grade</td>
<td>0.83</td>
<td></td>
<td>1.57</td>
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</tbody>
</table>

Table 2. Cartilage degeneration scores for the total pool of tali and for the subset displaying crystals.
MSU crystals (sometimes) induce inflammatory responses
Factors in joints such as FFA modulate MSU’s inflammatory response

Figure 1. MSU crystals do not induce IL-1β production or joint inflammation

FFAs may be the second hit in MSU-induced inflammation

Figure 2. MSU synergizes with FFA for cytokine production

Chondroitin sulfate suppresses MSU induced IL-1β production by macrophages

Orlowsky et al. BMC Musculoskeletal Disorders 2014, 15:318
Gout and OA

What we know
• MSU crystals damage cartilage
• OA predisposes to gout

What we don’t know
• Why urate deposits seem to become part of cartilage
• What initiates an acute inflammatory response
• Why crystals form
• One hit vs two hits
Calcium crystals

- Calcium Pyrophosphate (CPP)
- Basic calcium phosphate (BCP)
Calcium crystals are understudied
Calcium crystals are common in OA synovial fluids.
Calcium crystals are really common in OA joint tissues

OA severity correlates with the presence and quantity of calcium crystals

Calcium crystals contribute to cartilage damage through multiple mechanisms.
Excess extracellular pyrophosphate is necessary for CPP crystal formation

Is ANK the PPI transporter?
ANK mutations are linked to familial CPPD and excessive articular calcification.
Most PPi is generated by extracellular ATP

- Factors that increase levels of ePPi around chondrocytes also increase extracellular ATP.
- There is no known extracellular enzyme that regenerates ATP from PPi.
Over-expression of ANK increases extracellular ATP levels in chondrocytes
siANK reduces extracellular ATP levels in chondrocytes
Probenecid, an ANK inhibitor, reduces eATP levels
Matrix vesicles in growth plate cartilage mediate calcification
Analogous vesicles are present in articular cartilage

In situ EM

Isolated ACVs from cartilage

Articular cartilage vesicles generate both CPPD and BCP crystals in vitro.
ACV number increases in conjunction with autophagy
ACV number increases in conjunction with autophagy

A

<table>
<thead>
<tr>
<th></th>
<th>Rapamycin</th>
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</thead>
<tbody>
<tr>
<td>LDH (% control)</td>
<td>115.1 ± 11.6</td>
</tr>
<tr>
<td>MTT (% control)</td>
<td>102.6 ± 20.3</td>
</tr>
<tr>
<td>caspase-3 activity (% control)</td>
<td>115.1 ± 3.94</td>
</tr>
</tbody>
</table>

B

Beclin-1
LC3
GAPDH
Rapamycin

4 hr 6 hr 8 hr 12 hr

C

Beclin-1
LC3
β-actin
Rapamycin
4 hr 6 hr 8 hr 12 hr

D

ACVs (e^-9)

<table>
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<tr>
<th>hours post-treatment</th>
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<tbody>
<tr>
<td>4</td>
</tr>
<tr>
<td>ε^-9 high glucose</td>
</tr>
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</table>

ACV: Autophagy-Related Vesicles
ACV number increases in conjunction with autophagy
ACVs contain LC3-II and usual ectoenzymes and RNA
Autophagy increases ACV formation and release in chondrocytes.
Matrix matters!!
BCP crystal formation

- BCP crystals coexist with CPP crystals
- PPi/Pi ratio critically regulates which type of crystal forms.
  - Pi/PPi < 6 → CPPD
  - Pi/PPi > 140 → BCP
Meniscal calcification accelerates OA

From: Cheung et al, Arthritis Rheum 54:2452, 2006
Clinical association of vascular and cartilage calcification in CPPD suggests a systemic process

<table>
<thead>
<tr>
<th>Extra-articular sites with calcification</th>
<th>CC</th>
<th>OR (95% CI)</th>
<th>aOR (95% CI)</th>
<th>Number of joints with CC</th>
<th>OR (95% CI)</th>
<th>aOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>2372  324  1.00  1.00</td>
<td>1.00  1.00</td>
<td>193  131  1.00  1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Either vascular or soft-tissue calcification</td>
<td>306  88  2.11 (1.62 to 2.74)  1.85 (1.40 to 2.44)</td>
<td>48  40  1.23 (0.76 to 1.97)  1.12 (0.67 to 1.87)</td>
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</tr>
<tr>
<td>Vascular and soft-tissue calcification</td>
<td>23  13  4.14 (2.08 to 8.25)  3.08 (1.50 to 6.31)</td>
<td>9  4  0.66 (0.20 to 2.17)  0.48 (0.13 to 1.75)</td>
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CC, chondrocalcinosis.

Calcium crystals directly interact with synovial fibroblasts and chondrocytes
Calcium crystals (sometimes) induce an inflammatory response
Animal models are problematic in crystal diseases
Summary

• Crystals may cause and certainly worsen OA
• Calcium crystal-associated arthritis is under-studied and under-recognized.
• Animal models present unique challenges in crystal-induced arthritis.
• Crystals are interesting and important!!
Thanks to the VA Research Service, my lab, William T. Jackson, PhD, Department of Microbiology, MCW and Carolyn Coyne, PhD, University of Pittsburgh.