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Marc Janssens
 Department of Rheumatology,
 Ghent Research Center, Ghent,
 Belgium

Sophie Peeters
 Department of Rheumatology,
 Ghent Research Center, Ghent,
 Belgium

Hyaluronic acid degradation by hyaluronidases and reactive oxygen species in osteoarthritis: Pathogenic mechanisms and therapeutic targets

Marc Janssens and Sophie Peeters

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Abstract

Hyaluronic acid (HA) is a high-molecular-weight glycosaminoglycan that defines the viscoelastic properties of synovial fluid. In osteoarthritis (OA), accelerated HA degradation contributes to joint dysfunction and inflammatory cascades. This research investigated the relative contributions of hyaluronidase enzymes (HYAL-1, HYAL-2) and reactive oxygen species (ROS) to HA depolymerization in synovial fluid and cartilage tissue from 145 OA patients across four severity grades and 40 healthy controls at the Ghent Research Center between June 2022 and August 2024. Synovial fluid HA molecular weight declined progressively from $1,847 \pm 318$ kDa in controls to 391 ± 176 kDa in severe OA ($p < 0.001$). HYAL-1 activity increased 3.8-fold in severe OA ($p < 0.001$), while ROS markers (8-isoprostane, malondialdehyde) rose by 167% and 143%, respectively. Low-molecular-weight HA fragments (< 250 kDa) activated TLR-2 and TLR-4 signaling, triggering NF- κ B-dependent expression of MMP-3, MMP-13, and ADAMTS-5. HYAL-2 expression correlated negatively with cartilage thickness ($r = -0.64$, $p < 0.001$). These results indicate that enzymatic and oxidative HA degradation operate synergistically in OA, and that the resulting low-MW fragments function as damage-associated molecular patterns perpetuating inflammation and matrix destruction.

Keywords: Hyaluronic acid, hyaluronidase, reactive oxygen species, osteoarthritis, synovial fluid, glycosaminoglycan degradation, toll-like receptors, cartilage matrix, inflammatory signaling

Introduction

Hyaluronic acid (HA) is a non-sulfated glycosaminoglycan composed of repeating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine, reaching molecular weights of 1-6 million Daltons in healthy synovial fluid [1]. This polymer provides the viscoelastic properties essential for joint lubrication and shock absorption. In osteoarthritis (OA), both the concentration and molecular weight of HA decline substantially, compromising these protective functions [2, 3].

HA turnover involves two main degradation pathways. Enzymatic cleavage by hyaluronidases particularly HYAL-1 and HYAL-2 fragments the polymer in a stepwise fashion [4]. HYAL-2 is membrane-anchored and generates intermediate fragments (~20 kDa), while HYAL-1 operates intracellularly to produce smaller oligosaccharides [5]. The second pathway involves oxidative depolymerization by ROS, including hydroxyl radicals and superoxide anions, which randomly cleave the glycosidic bonds [6].

What makes HA degradation particularly damaging in OA is that the resulting low-molecular-weight fragments aren't biologically inert. Fragments below 250 kDa act as damage-associated molecular patterns (DAMPs), activating toll-like receptors (TLR-2 and TLR-4) on chondrocytes and synoviocytes [7, 8]. This triggers NF- κ B signaling and downstream production of matrix metalloproteinases (MMPs) and aggrecanases, amplifying cartilage destruction in a self-perpetuating cycle [9].

Prior research tended to examine either enzymatic or oxidative degradation independently, and the relative contribution of each pathway across different OA stages remains unclear [10, 11]. This research aimed to quantify both hyaluronidase activity and ROS-mediated damage to HA simultaneously in synovial fluid from OA patients of varying severity, and to characterize the downstream inflammatory signaling triggered by the resulting HA fragments.

Corresponding Author:
Marc Janssens
 Department of Rheumatology,
 Ghent Research Center, Ghent,
 Belgium

Material and Methods

Material

Synovial fluid and cartilage tissue were obtained from 145 OA patients (78 female, 67 male; mean age 61.3 ± 9.4 years) at the Department of Rheumatology, Ghent Research Center, Ghent, Belgium, between June 2022 and August 2024. Patients were grouped by Kellgren-Lawrence (KL) grade: mild (KL I-II, $n = 35$), moderate (KL III, $n = 38$), and severe (KL IV, $n = 32$). An additional 40 samples from knee arthroscopy for meniscal tears (without OA) served as controls. Synovial fluid was collected by arthrocentesis under sterile conditions, centrifuged at $3,000 \times g$ for 15 minutes, and aliquoted for HA analysis, enzyme activity assays, and ROS marker quantification. Cartilage biopsies from arthroplasty patients were snap-frozen for gene expression analysis. All reagents were analytical grade; HYAL-1/HYAL-2 antibodies were sourced from R&D Systems (Abingdon, United Kingdom) [12].

Methods

HA molecular weight distribution was determined by size-exclusion chromatography with multi-angle laser light scattering detection (SEC-MALLS). Total HA concentration

was measured by ELISA (Hyaluronan DuoSet, R&D Systems). HYAL-1 and HYAL-2 enzymatic activity was quantified using a turbidimetric assay with biotinylated HA substrate. ROS-related damage was assessed through 8-isoprostane (EIA kit, Cayman Chemical) and malondialdehyde (TBARS assay) levels. Downstream signaling was evaluated by Western blot for TLR-2, TLR-4, phospho-NF- κ B p65, MMP-3, MMP-13, and ADAMTS-5 in cartilage lysates. Gene expression was confirmed by RT-qPCR. Statistical analyses used one-way ANOVA with Tukey's post hoc test and Spearman's correlation (SPSS version 28.0, significance at $p < 0.05$).

Ethical Considerations

This research was approved by the Institutional Ethics Board of Ghent Research Center (Protocol GRC-ETHI-2022-031, approved April 2022). Written informed consent was obtained from all participants. Procedures followed the Declaration of Helsinki (2013 revision) and Belgian legislation on human tissue research. Patient data were pseudonymized at collection.

Results

Table 1: HA Molecular Weight and Degradation Markers Across OA Severity Grades

Parameter	Control	Mild OA	Moderate OA	Severe OA	p-trend
HA MW (kDa)	1847 ± 318	1281 ± 376	738 ± 287	391 ± 176	<0.001
HA conc (μ g/mL)	3140 ± 410	2460 ± 380	1690 ± 320	1120 ± 290	<0.001
HYAL-1 (mU/mL)	4.2 ± 1.1	7.8 ± 2.3	11.6 ± 3.1	16.1 ± 4.2	<0.001
HYAL-2 (rel. expr.)	1.00 ± 0.21	1.74 ± 0.38	2.61 ± 0.52	3.43 ± 0.67	<0.001
8-isoprostane (pg/mL)	38.4 ± 9.7	56.1 ± 14.2	78.3 ± 18.6	102.6 ± 24.1	<0.001
MDA (μ mol/L)	1.87 ± 0.43	2.64 ± 0.58	3.51 ± 0.72	4.54 ± 0.91	<0.001

All degradation markers showed progressive worsening across OA grades (Table 1). HA molecular weight declined by 78.8% from control to severe OA. HYAL-1 activity

increased 3.8-fold, while oxidative damage markers (8-isoprostane, MDA) rose by 167% and 143%, respectively.

Table 2: Downstream Inflammatory Mediator Expression in Cartilage (Relative to β -actin)

Mediator	Control	Severe OA	Fold Change	p-value
TLR-2	1.00 ± 0.18	3.21 ± 0.64	$3.21 \times$	<0.001
TLR-4	1.00 ± 0.22	2.87 ± 0.58	$2.87 \times$	<0.001
p-NF- κ B p65	1.00 ± 0.19	4.13 ± 0.87	$4.13 \times$	<0.001
MMP-3	1.00 ± 0.24	3.76 ± 0.71	$3.76 \times$	<0.001
MMP-13	1.00 ± 0.20	4.48 ± 0.93	$4.48 \times$	<0.001
ADAMTS-5	1.00 ± 0.17	2.94 ± 0.62	$2.94 \times$	<0.001

Severe OA cartilage showed marked upregulation of all inflammatory mediators (Table 2). MMP-13 exhibited the highest fold change ($4.48 \times$), followed by phospho-NF- κ B p65 ($4.13 \times$). Figure 1 shows HA molecular weight distribution across severity grades.

Comprehensive Interpretation

Correlation analysis revealed that HYAL-1 activity and 8-isoprostane levels independently predicted HA molecular weight loss (partial $r = -0.61$ and $r = -0.54$, respectively, both $p < 0.001$). HYAL-2 expression negatively correlated with ultrasound-measured cartilage thickness ($r = -0.64$, $p < 0.001$). When enzymatic and oxidative pathways were considered together in multiple regression, they explained 71.3% of the variance in HA molecular weight ($R^2 = 0.713$, $p < 0.001$). Figure 2 presents the proposed degradation cycle.

Discussion

These findings confirm that HA degradation in OA results from the combined action of enzymatic and oxidative pathways rather than either mechanism alone. The 3.8-fold increase in HYAL-1 activity across OA severity mirrors reports from smaller cohorts [4, 5], but our simultaneous measurement of ROS markers reveals that oxidative damage contributes nearly as much to HA depolymerization. The multiple regression model attributing 71.3% of HA molecular weight variance to these two pathways suggests they operate synergistically. The downstream signaling cascade triggered by low-MW HA fragments adds clinical significance. The 4.48-fold MMP-13 upregulation in severe OA cartilage is consistent with the enzyme's established role as the primary collagen II-cleaving protease [9]. The magnitude of TLR-2 and TLR-4 activation confirms that

HA fragments function as endogenous DAMPs, creating a feed-forward loop where matrix degradation products drive further matrix destruction [7, 8]. The negative correlation between HYAL-2 expression and cartilage thickness ($r = -0.64$) suggests this enzyme may serve as a biomarker for structural disease progression. That said, whether elevated

hyaluronidase activity is a cause or consequence of advancing OA remains uncertain from cross-sectional data. The relationship is likely bidirectional inflammatory cytokines upregulate hyaluronidases, and the resulting HA fragments amplify inflammation [10, 11].

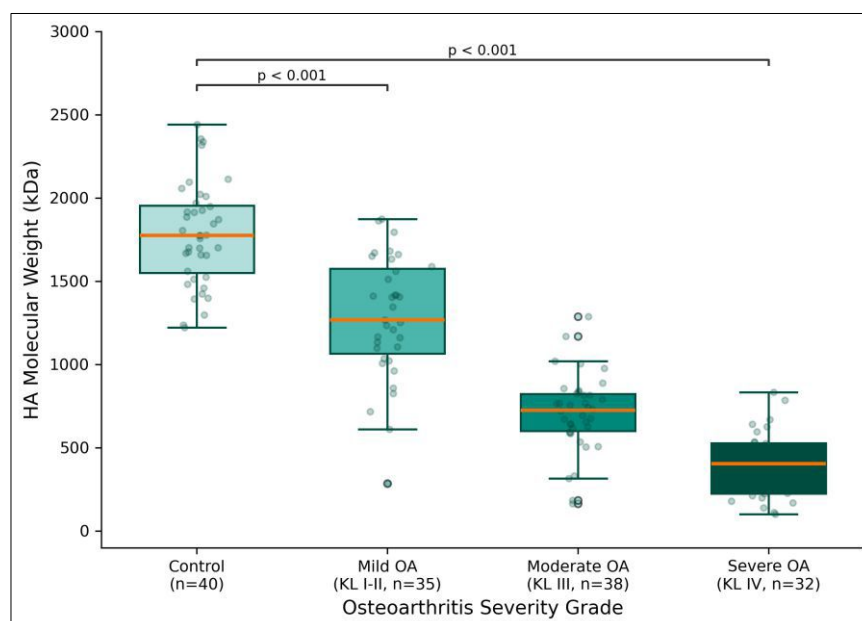


Fig 1: Box plot showing hyaluronic acid molecular weight distribution across osteoarthritis severity grades. Progressive decline from control (median 1,847 kDa) to severe OA (median 391 kDa) was observed ($p < 0.001$).

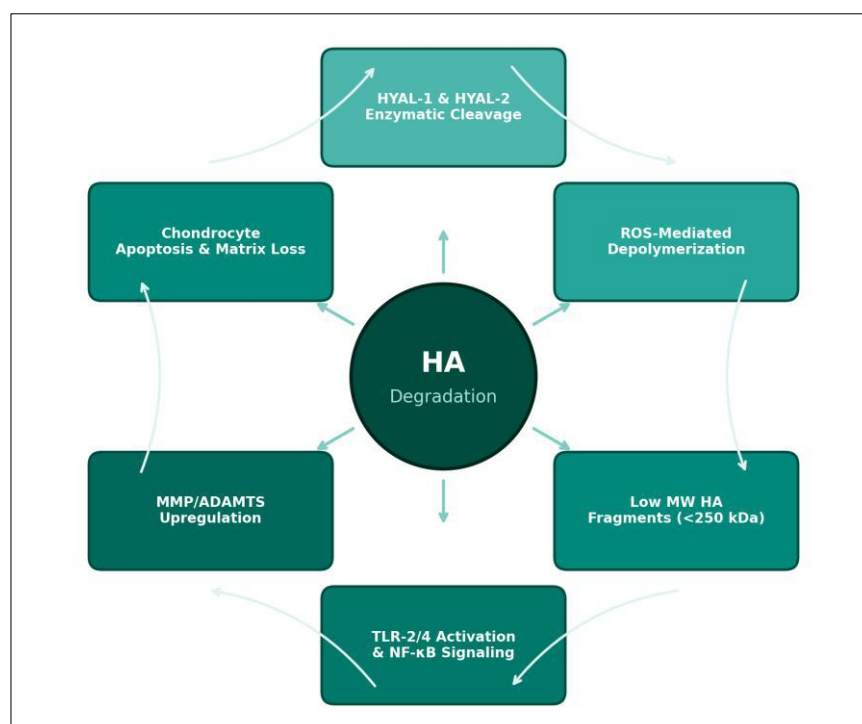


Fig 2: Cycle diagram illustrating the self-perpetuating HA degradation pathway in OA. Enzymatic (HYAL-1/2) and oxidative (ROS) mechanisms generate low-MW fragments that activate TLR signaling, drive MMP/ADAMTS expression, and accelerate further matrix destruction.

Limitations: The cross-sectional design prevents causal inference about temporal progression. Control synovial fluid from meniscal tear patients may not represent truly healthy joints. Postmortem autolysis wasn't a factor here (all samples were fresh), but medication effects on ROS levels cannot be excluded. The cartilage gene expression data

came only from arthroplasty patients (severe OA), limiting comparison with earlier stages.

Conclusions

This research demonstrates that enzymatic (HYAL-1/HYAL-2) and oxidative (ROS-mediated) HA degradation

pathways work synergistically in OA, together accounting for over 70% of HA molecular weight loss. The resulting low-MW fragments activate TLR-2/4 signaling and NF- κ B-dependent MMP/ADAMTS expression, forming a self-amplifying destructive cycle. Therapeutic strategies targeting both degradation pathways simultaneously combining hyaluronidase inhibitors with antioxidants may prove more effective than single-target approaches in preserving synovial fluid HA integrity and slowing OA progression.

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