Life Sciences xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Hyaluronan in experimental injured/inflamed cartilage: In vivo studies

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ARTICLE INFO

Keywords:
Hyaluronan
NF-kB
TLR-4
Cytokines
Joint inflammation
Osteoarthritis
Arthritis

ABSTRACT

Joint disease is characterized by an imbalance between the synthesis and degradation of articular cartilage and subchondral bone accompanied by capsular fibrosis, osteophyte formation and varying degrees of inflammation of the synovial membrane. Many animal models have been developed to study arthritis and osteoarthritis that enable experimental conditions, diet and environmental risk factors to be carefully controlled. Animal-based studies have demonstrated the positive effects of exogenous HA on the preservation of joint cartilage in different models of arthritis and osteoarthritis. Although many promising effects of exogenous HA have been reported, there remains uncertainty as to its effectiveness in reversing cartilage injury and other manifestations of joint diseases because of difficulties in interpreting and unifying the results of these studies.

A review of the literature of the last decade was conducted to report the results and to determine what we have learned from animal models in relation to joint inflammation induced by experimental models and HA treatment.

1. Introduction

The most common joint damage suffered worldwide is the erosion and destruction of joint cartilage caused by RA and OA, which causes pain and physical disability in the aging population [1,2]. Inflammation mediated by pro-inflammatory cytokines is responsible for cartilage degradation that most commonly affects the knees, hands, feet, hips and spine. In synovial joints, the entire joint is affected, including cartilage, the synovial membrane, subchondral bone, ligaments and periarticular muscles [1,2]. Several recent findings have provided clear evidence of the detrimental role of inflammation in OA and RA, suggesting that inflammatory intermediates contribute to the symptoms and the progression of these diseases [3,4]. The most common clinical symptoms are joint pain connected to use, crepitus during movement due to tissue erosion and joint stiffness from short-lasting inactivity [5]. It has been reported that the inflammatory changes found in inflamed joints commonly occur in the synovial lining, where an increased number of macrophages are activated [6,7]. HA is a non-sulfated component of the ECM that is also abundant in the synovial fluid. Exogenous HA is often used in clinical practice to treat joint inflammation. HA functions as a cartilage joint lubricant during movement and acts as a damper during compression. In pathologies affecting joints, a substantial decrease in the concentration and molecular size of HA significantly reduces the viscoelastic features of synovial fluid and triggers a strong inflammatory response [8]. HA is injected exogenously in order to replace and stimulate degraded endogenous HA, as well as to mitigate the inflammatory process [9]. HA can ameliorate the symptoms of diseased joints through different mechanisms including: mechanical

Abbreviations: ACAN, aggrecan; ADAMTS-5, A disintegrin and metalloproteinase with thrombospondin motifs 5; $A2_AR$, adenosine type 2 receptor; Bax, BCL2 associated X protein; Bcl-2, B-cell lymphoma 2; CD44, cluster determinant 44; Col2A, collagen type II alpha; Col1a1, collagen type I alpha1; Col2a1, collagen type II alpha1; Col3a1, collagen type III alpha1; Col5a1, collagen type V alpha1; Col10a1, collagen type X alpha1; CTGF, connective tissue growth factor; ECM, extracellular matrix; GAGs, glycosaminoglycans; HA, hyaluronan; HASs, hyaluronan synthases; HAS1, HAS2, HAS3, hyaluronan synthase 1,2,3; HSP-47, heat shock protein-47; HSP-70, heat shock protein-70; HYALs, hyaluronidases; HYAL-1, hyaluronidase-1; HYAL-2, hyaluronidase-2; IL-1β, interleukin-1β; IL-6, interleukin-6; IL-17, interleukin-17; iNOS, inducible nitric oxide synthase; kDa, kiloDaltons; LOX, lipoxygenase inhibitor; MMPs, metalloproteinases; MMP-1, MMP-3, MMP-13, metalloproteinase-1,3,13; mRNA, messenger of ribonucleic acid; 4-MU, 4-methylumbelliferone; MyD88, myeloid differentiation primary response gene 88; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; NO, nitric oxide; NSAIDs, nonsteroidal anti-inflammatory drugs; OA, osteoarthritis; PCR, polymerase chain reaction; PGE2, prostaglandin E-2; PGs, proteoglycans; PRP, platelet-rich plasma; RA, rheumatoid arthritis; ROS, reactive oxygen species; SF, synovial fibroblasts; β-TCP, β-tricalcium phosphate; TGF-β, transforming growth factor beta; TGF-β1, transforming growth factor beta-1; TLR2, TLR4, toll-like receptor-2,4; TNF-α, tumor necrosis factor-α; TRAF6, TNF receptor associated factor-6

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https://doi.org/10.1016/j.lfs.2017.11.006
Received 11 September 2017; Received in revised form 28 October 2017; Accepted 7 November 2017

viscosupplementation of damaged joints with consequent re-establishment of joint homeostasis by inducing endogenous HA production; inhibition of inflammatory pathways thus reducing degradative enzymes and pro-inflammatory mediators; stimulation of cartilage anabolism and by reducing free radical production [10,11].

There is substantial evidence to suggest that HA action differs in relation to its molecular mass. In fact, there is a great deal of experimental data demonstrating that HA size may affect its efficacy and safety, the conclusion being that the use of HA at high molecular mass is more efficient than HA at low molecular mass [12]. However, many of these studies are heterogeneous, particularly as regards the HA type used, and the conclusions drawn are often not supported by other studies.

Mechanical studies on molecular HA action have shown the anti-inflammatory activity of HA at high molecular weight, whereas low molecular weight HA (generated by the degradation of native HA) is able to induce a strong inflammatory response [12]. In vitro studies have reported that small HA fragments may produce a range of pro-inflammatory responses, including the activation of murine alveolar macrophages and the maturation of human dendritic cells [13,14]. Other investigations have reported the activity of several pro-inflammatory cytokines and other detrimental intermediates to be increased by small HA oligosaccharides [15,16,17,18]. A number of experimental studies have confirmed CD44, TLR2, and TLR4 as the candidates able to activate this series of events in immune system cells through HA signaling [15,19,20,21]. Many in vitro studies, using immune cells from injured tissue, have demonstrated low molecular weight HA to be responsible for TLR2 and TLR4 activation by initiating the MyD88-dependent NFκB signaling cascade and by pro-inflammatory cytokine gene expression [20,22]. In addition, small HA oligosaccharides have been found to stimulate both CD44 and TLRs, thereby activating NF-κB with the consequent transcription of a large number of pro-inflammatory cytokines and other active mediators that induce cell damage (Table 1) [23,24]. By contrast, high molecular weight HA in its native form as a large polymer exhibits anti-inflammatory and immunosuppressive properties by reducing these inflammatory mediators (Table 2). The anti-inflammatory potential of HA at high molecular weight has been well-documented using different models of experimental inflammation [25]. Since several authors have reviewed the effects of HA at different molecular mass in chondrocytes and other cultured cells, here we have sought to review the most recent advances related to the use of HA in experimental animal models in which RA or OA were induced so that these results may aid future research in this field.

2. HA features

HA was first isolated in 1934 from the vitreous body of cow eyes. This newly discovered compound was found to contain two sugar molecules, one of which was uronic acid. The popular name hyaluronic acid was adopted since it is derived from “hyalos”, which is the Greek word for glass, plus uronic acid [26]. At the time, nobody knew that it would prove to be one of the most important and useful natural polymers, widely used in medicine to our day. HA was marketed for the first time in 1942 when it was used as a substitute for egg white in baked goods [27]. The current name hyaluronan was introduced in the year 1986 to conform to the international nomenclature of polysaccharides [28]. Therefore the different terms for HA derive from the different forms of the molecule; the acid form is called hyaluronic acid while the salt, sodium hyaluronate, is called hyaluronan. In the past, HA was obtained from various sources and its physicochemical structure, properties and biological role rigorously investigated all over the world [28]. HA is a compound belonging to a large family of GAGs, which are the principal components of the ECM. HA differs from other GAGs in terms of its simple structure and high molecular mass [29]. HA structure is composed of a long series of disaccharide units, each formed by D-glucuronic acid and *N*-acetyl-D-glucosamine linked by β-glycosidic bonds [29]. Both these sugars are spatially related to glucose, which in the β-configuration allows all its bulky groups (the hydroxyls, the carboxylate portion, and the anomeric carbon on the adjacent sugar) to be in sterically favored equatorial positions while all of the small hydrogen atoms occupy the less sterically favored axial positions in order to achieve the most stable configuration; the structure of the disaccharide is therefore energetically highly stable [30]. Repeating the basal unit of HA thousands of times forms a lengthy linear polymer, with a molecular weight of up to 6000 kDa [30]. In physiological solution, the backbone of the HA molecule is present as a rigid complex due to the combined effect of the disaccharide chemical structure, internal hydrogen bonds and interactions with the water present. Notably, the axial hydrogen atoms form a non-polar, relatively hydrophobic face while the equatorial side chains form a more polar, hydrophilic face, thereby producing a twisted filament structure. HA solutions have outstanding rheological properties and extraordinary lubricant and hydrophilic characteristics. When solubilized in water, HA adopts the form of an expanded random coil. At higher concentrations it confers extremely high viscosity to solutions. When HA is dissolved at 1.0%, commonly in water, it aggregates into a jelly-like state. However, when HA is compressed, such as in a syringe, it can be routinely administered through a small needle. It is also used as a natural pseudo-plastic compound. The extraordinary rheological properties of HA solutions make them ideal natural lubricants for eroded joints. Several experimental findings have shown the ability of HA to separate most tissue surfaces that slide along each other. The high lubricant capacity of HA resulting from the formation of a coil structure capable of trapping about 1000 times its weight in water has been demonstrated to limit postoperative adhesion formation after orthopedic surgery [27,30].

HA is synthesized by HASs, a family of three HA synthases (HAS1, HAS2, HAS3) that are multipass transmembrane enzymes on the inner surface of the cellular membrane. When released into extracellular space the polymeric chain lengthens. Therefore HA synthesis differs

Table 1
Biological effects of degraded HA.

Effects	Reference
Increase of inflammation mediators	[8,12,15–21,23–25,48,55,59]
Increase of degrading enzymes	[17,20,48]
Increase of HASs and iNOS	[17,20,24,48]
Activation of dendritic cells	[13,14]
Activation of TLR-2/4	[13,15–21,23–25,48,55]
Activation of CD44	[13,15,17–21,23–25,55]
Activation of apoptosis	[23,59]
Activation of NF-kB	[15–25,48,55]

Table 2
Biological effects of high polymerized HA.

Effects	Reference
Anti-inflammatory and immunosuppressive action	[9,10,16,19–23,25,38,40,42,45,47–49,55]
Cellular growth/proliferation	[23,36,37,39,45,46]
Inhibition of activated NF-kB	[16,19–23,25]
Inhibition of ROS production	[11,63]
Inhibition of apoptosis	[19,43,59,63]
Increase in ECM component synthesis and cartilage protection	[23,27,30,35–37,39–42,44–50,52–54,56–64]
Block of TLR/4 and/or CD44 receptors	[16,19–22,25,48,50,55]
Inhibition of pro-inflammatory mediators and degrading enzymes production	[16,19–23,25,38,40,42,45,47,48,51,55,59]