Antioxidant Profile of Hyaluronan: Physico-Chemical Features and its Role in Pathologies

G. Mendoza, J.G. Prieto, R. Real, M. Pérez, G. Merino and A.I. Álvarez*

Department of Biomedical Sciences, University of Leon, E-24071 Leon, Spain

Abstract: Many findings have evidenced antioxidant properties of hyaluronan, both *in vitro* and *in vivo*, by means of which it can scavenge free radicals and exert its effect on pathologies. The aim of this review is to summarize the available data on the features and clinical profile of hyaluronan, with regard in particular to its antioxidant capacity and to its related physico-chemical properties. Additionally, hyaluronan and its derivatives are examined, with the focus on their therapeutic uses, protection against cellular damage, and their role as inflammatory mediators. Finally, therapies associated to the antioxidant effect of hyaluronan are discussed.

Key Words: Hyaluronan, antioxidants, reactive oxygen species, oxidative damage, inflammation, therapeutic use.

INTRODUCTION

Hyaluronan (HA) is a high molecular-weight (MW), highly anionic glycosaminoglycan (GAG), which appears ubiquitously distributed in vertebrates' tissues, being most abundant in the extracellular matrix of soft connective tissues [1].

HA can interact with enzymatic and chemical systems, with important consequences in the cellular environment [2]. HA acts as an antioxidant since it is a substrate of ROS and reduces the production of ROS by chelating metal cations [3].

In spite of its simple chemical structure, HA is a fascinating macromolecule due to its physiological effects and its role in a wide range of pathologies, both at molecular and cellular levels. Its involvement and its effects in such a variety of events are a direct consequence of its physicochemical properties, which are highly important for its biomedical and biotechnological applications.

In this review we briefly summarize and update data on the application or the potential benefits of HA and its derivatives, from a chemical point of view.

PHYSICOCHEMICAL PROPERTIES OF HA

HA is a linear high MW non-sulfated natural polysaccharide comprised of strictly alternating sequences of 3-linked 2-acetamido-2-deoxy-β-D-glucopyranose and 4-linked β-D-glucuronic acid residues (Fig. (**1**)). Its MW is typically between 0.2-100 MDa. It is synthesized at the inner face of the plasma membrane as a free linear polymer without any protein core [4]. It usually appears as a polyanion, not as a free acid.

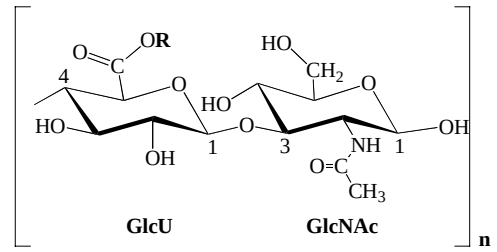
Despite its relatively simple structure, HA behaves as an unusually stiff polymer in solution. Local conformation is, on average, that of an approximately 4-fold helix with disaccharide as the fundamental unit. The weak and transient hydrogen bonds within its structure appear to allow rapid interchange with local water molecules at glycosidic bonds level, which lends some stability to the HA molecule in solution as well as substantial local dynamics. The involvement of water molecules gives the linkage more flexibility, and can engender relatively large and rapid changes in conformation [5].

Among HA physicochemical characteristics, viscoelastic and rheological properties are the most relevant and support most of its physiological and therapeutic functions [5,6]. The rheological behavior is of decisive importance for almost all applications, and the MW of the molecule emphazises its viscosity. Furthermore, in aqueous solutions HA shows high viscoelastic behavior and water binding capacity due to the high MW and the high number of polar, charged groups [6].

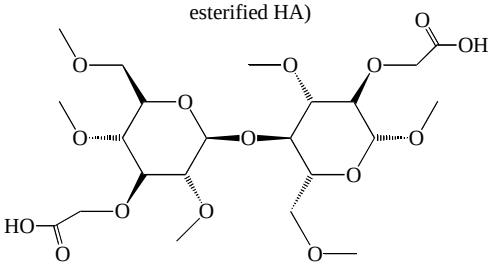
In this sense, rheological and scattering studies have concluded that HA chains are semiflexible in solution. HA is therefore an excellent space-filling molecule that can undergo deformation as required during rapid growth and tissue remodelling, while entrapping water and ions to maintain tissue hydration and buffer the local environment [7]. Its viscoelasticity allows it to move unhindered into vacant spaces where it can keep cells partially localized and give them a substrate on which to move. There is little evidence for specific interactions between chains in saline solution, suggesting the lack of a firmly established HA network under these conditions, which would be stabilized by chain entanglement. On the other hand, the enormous HA hydrodynamic volume and the simple and transient intermolecular interactions, which are more common with the superposition of molecular domains, determine HA viscosity [8]. HA is synthesized by HA synthases bounded to the plasma membrane of bacteria and eukaryotic cells. It is a dynamic and continuous process, just like catabolism. HA degradation is also sustained, yielding an extraordinarily rapid turnover of HA in vertebrates. HA degradation *in vivo* can proceed by two main, and simultaneous, mechanisms: enzymatic degradation and chemical depolymerization.

*Address correspondence to this author at the Department of Biomedical Sciences, University of Leon, E-24071 Leon, Spain; Tel: +34 987 291 265; Fax: +34 987 291 267; E-mail: ana.alvarez@unileon.es

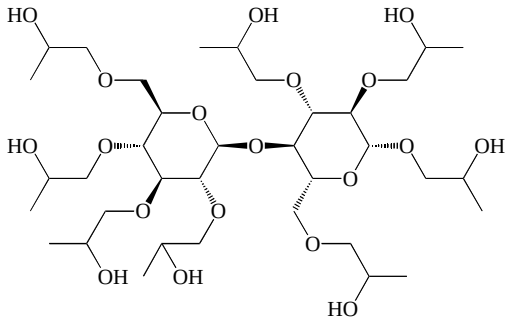
1389-5575/09 $55.00+.00



HA and esterified HA (R =H in HA; R = other compounds in esterified HA)



Carboxymethylcellulose



Hydroxypropylmethylcellulose

Fig. (1). Chemical structure of hyaluronan (acid form), esterified hyaluronan, carboxymethylcellulose and hydroxypropylmethylcellulose. GlcA= Glucuronic Acid; GlcNAc = N-Acetyl-Glucosamine.

HA enzymatic degradation is a step-wise process performed by hyaluronidases, some of their isoforms being functional not only intracellularly but also extracellularly. In the ECM of most mature tissues, HA has a high MW. Together with other structural macromolecules, HA contributes to the mechanical properties of the meshwork. Hence, in order to be released from this firm network, the polymer has to be at least partially degraded. Subsequently, the cells can take up intermediate-sized chains either by receptor-mediated mechanisms or by endocytosis. In consequence, HA is sorted into the lysosomal compartment, where it becomes hydrolyzed to small oligosaccharides by intracellular hyaluronidases. These fragments are eventually degraded to monosaccharides by exoglucosidases present in lysosomes [9].

However, in HA catabolism reactive oxygen species (ROS) also take part. In some organs and in synovial fluid, hyaluronidases appear at very low concentrations, in spite of its rapid turnover, so it is accepted that HA chemical catabolism is a well-established process in joints [10]. In some pathologies, such as acute inflammatory conditions or tissue damage, HA catabolism by ROS is a common process [11]. In inflammation, the release of chemical mediators, i.e. ROS, is the first stage of the inflammatory response. These reactive species can lead to oxidative stress, which means the alteration of the intracellular redox homeostasis, resulting in a serious imbalance between production of reactive species and antioxidant defense [12]. This alteration leads to damage in a wide range of structures and molecules, including lipids, proteins and nucleic acids [13]. The excessive amount of ROS in inflammatory conditions also affects HA integrity, resulting in its degradation. This depolymerization depends on the type of ROS that attacks HA. For instance, superoxide radicals ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2) seem to produce other more reactive species which depolymerize HA by different mechanisms [14]; $O_2^{\cdot -}$ also works together with other ROS, like HClO [15]; and hydroxyl radicals ($^{\cdot}OH$), mainly formed by a Fenton, or an analogous, reaction [16], attack glucuronic acid subunits, breaking the stability of the molecule and generating final open-chain products [17], which leads to a reduction in HA MW [18].

Degradation products obtained from enzymatic or chemical mechanisms highlight the differences between both processes. Though the result of both processes is the destabilization of the HA chemical structure and its depolymerization, hyaluronidase attack yields fragments with identical structure of the parent chain, while fragments generated by chemical attack differs from the structure of the parent polymer.

HA ANTIOXIDANT CAPACITY

Halliwell and Gutteridge [19] define antioxidant as any substance that, when present in low concentrations compared to that of an oxidisable substrate, significantly delays or inhibits the oxidation of that substrate. Physiologically, the role of antioxidants is to prevent damage to cellular components arising as a consequence of chemical reactions involving free radicals.

As we described above, HA is susceptible of ROS attack, which leads to changes in its structure and its degradation, modulating oxidative damage [20]. Recent literature reveals a relation between HA depolymerization by ROS and its capacity to scavenge these species, and therefore, exert its antioxidant ability [21-23].

In this sense, HA capacity to scavenge $^{\cdot}OH$, peroxynitrite (ONOO–), $O_2^{\cdot -}$ and peroxyl radicals in a dose-dependent manner has been demonstrated [21]. Among ROS, $^{\cdot}OH$ is the most effective species in HA degradation, which is in agreement with the studies that show this ROS as responsible for HA depolymerization in inflammation [2,24-29]. Besides the direct HA antioxidant effect, other authors have highlighted the importance of the HA antioxidant role based on the chelation or elimination of Fe^{2+}, as well as the removal of proteins bound to Fe^{2+} [19,23,30]. Both processes are important in the protection against cell damage.

The relation between HA antioxidant ability and its MW [3,22] is specially interesting. The effect of $^{\cdot}OH$ and $O_2^{\cdot -}$ produced *in vitro* by no cellular systems or by polymorphonuclear leukocytes points to the higher antioxidant capa-

bility of high MW HA (3-6 MDa), which also exerts a concentration-dependent role, as compared to low MW HA (0.3 MDa). In fact, HA antioxidant properties are attributed to the formation of a viscous, pericellular meshwork around cells that restricts ROS movement in close proximity with cells or other biomolecules. The ability of HA to achieve this function has been shown to be MW- and concentration-dependent, as high MW HA forms a more effective exclusion zone than low MW HA [3,22,31-33]. MW is also a key factor in the ability of HA to directly compete with other cellular and molecular targets for ROS attack, with the secondary radical formed being confined within the HA structure, thus preventing attack of alternative sites. During such antioxidant activity, HA is known to lose viscosity, and undergo modification and chain depolymerisation [22].

HA DERIVATIVES AND THEIR ANTIOXIDANT PROPERTIES

HA association with metal ions, molecules or compounds involves modification of its chemical structure and, therefore, changes in its properties as antioxidant. Moreover, the concomitant use of HA with other antioxidants has revealed the protective role of these antioxidants in HA depolymerization by ROS.

Due to the presence of free carboxyl groups on glucuronic units, HA has a polyanionic character that allows the formation of associates with cations. Because of the potential advantageous effect of the different cations, several HA-counterion compounds have been prepared for medical purposes [34-38]. The most common HA associates in intraarticular injection solutions for osteoarthritis (OA) therapy are sodium HA. Bivalent cations, such as Zn^{2+}, Co^{2+} and Cu^{2+}, have a protective effect against ROS-induced HA degradation. It has been reported that these compounds are more effective antioxidants than HA [21], though the mechanisms by which they can exert antioxidant properties are not fully understood.

Other studies have pointed to the antioxidant activity of HA derivatives used in wound healing and ophthalmology, such as hylans (HA further enhanced by cross-linking) [39]; HA derivatives which comprise other compounds (methylprednisolone, hydrocortisone, steroids, alcohols) via esterification of the carboxyl groups in glucuronic acid residues [3,22]; and compounds composed of HA and carboxymethylcellulose [3] and of HA and hydroxypropylmethylcellulose [40] (Fig. (**1**)).

Antioxidant capacity of hylans has been highlighted, mainly against $^{\cdot}OH$, as well as its ability to inhibit $O_2^{\cdot -}$ generation by polymorphonuclear leukocytes [39].

Esterified HA also exerts antioxidant properties against $^{\cdot}OH$ and $O_2^{\cdot -}$ produced by no cellular *in vitro* systems [3] and by polymorphonuclear leukocytes [22]. Antioxidant capacity of this type of compounds is based on the aromatic groups contained within their structure, which scavenge ROS and act as chain-breaking antioxidants [41]. So esterification of benzyl groups offers numerous aromatic targets to compete for $O_2^{\cdot -}$ compared to HA.

Compounds built of HA and carboxymethylcellulose show higher antioxidant capacity than HA [3], just like a viscosurgical device based on sodium HA and hydroxypropylmethylcellulose, revealing the protective effect of hydroxypropylmethylcellulose on HA [40].

The studies cited have highlighted HA antioxidant capacity through its degradation by ROS, which is dependent on the HA derivative tested. These properties are highly important in therapeutic applications of these compounds. However, another scene opens up with the concomitant use of well-known antioxidants and HA, giving HA protection against ROS attack under e.g. inflammatory conditions, maintaining the structure and allowing HA to continue exerting its physiological and therapeutic functions, such as a viscoelastic device in viscosurgery and viscosupplementation.

In this respect, several authors have demonstrated the effect of a wide range of antioxidants which can protect HA against oxidative damage. A list of antioxidants including their chemical structure is depicted in Fig. (**2**).

Among antioxidants, probably the most tested, in relation to HA protection against ROS, is mannitol [42-46]. This polyol has been studied in different ROS generating systems, e.g. in a myeloperoxidase-H_2O_2 system [42], with $ONOO^-$ [43], H_2O_2 [45], and by an analogous Fenton reaction in which Cu^{2+} and H_2O_2 were involved [43,44,46]. In all these experiments, mannitol exerted an antioxidant role in a concentration-dependent manner, protecting HA against ROS depolymerization. However, its effects were quite different, showing an inhibition of 50% from 2 mM [42,44] to 20-26 mM [43,46], regardless of the ROS generating system.

Thiourea is another efficient antioxidant in HA protection [43,46], with different results from a maximum inhibition of 27% [46] to a total inhibition (no degradation) at a concentration of 2 mM [43]. Vinpocetine is an indole derivative with antioxidant properties; its highly protective role has been shown in HA degradation, inhibiting $^{\cdot}OH$ attack to 50% at concentrations lower than 2 μM [44,46]. Propofol, a widely used anaesthetic agent, also develops antioxidant activity against $^{\cdot}OH$ [46,47], as well as stobadine [44], D-penicillamine [48], superoxide dismutase (SOD) [42,49], desferrioxamine, histidine, and iodide and bromide ions [42].

Various anti-inflammatory agents have shown a clear protective role of $^{\cdot}OH$-mediated HA degradation [42,50,51]. A comparative study of the inhibition of the viscosity-decreasing effect of a myeloperoxidase degrading system of HA [42], revealed that paracetamol, lidocaine, salicylic acid, *p*-hydroxybenzoic acid, ibuprofen, and acetylsalicylic acid exert antioxidant properties in this order, from higher protective effect to lower. Other studies [50] have shown the inhibitory effects of naproxen and acetylsalicylic acid in $^{\cdot}OH$-mediated HA damage, revealing a concentration-dependent effect and a higher antioxidant role of naproxen than acetylsalicylic. The study of $^{\cdot}OH$ scavenging activity of ibuprofen enantiomers has highlighted the dose-dependent protective role against HA degradation, demonstrating that R-ibuprofen has slightly greater activity than the drug S-enantiomer [51]. The effect of these anti-inflammatory agents inhibiting HA

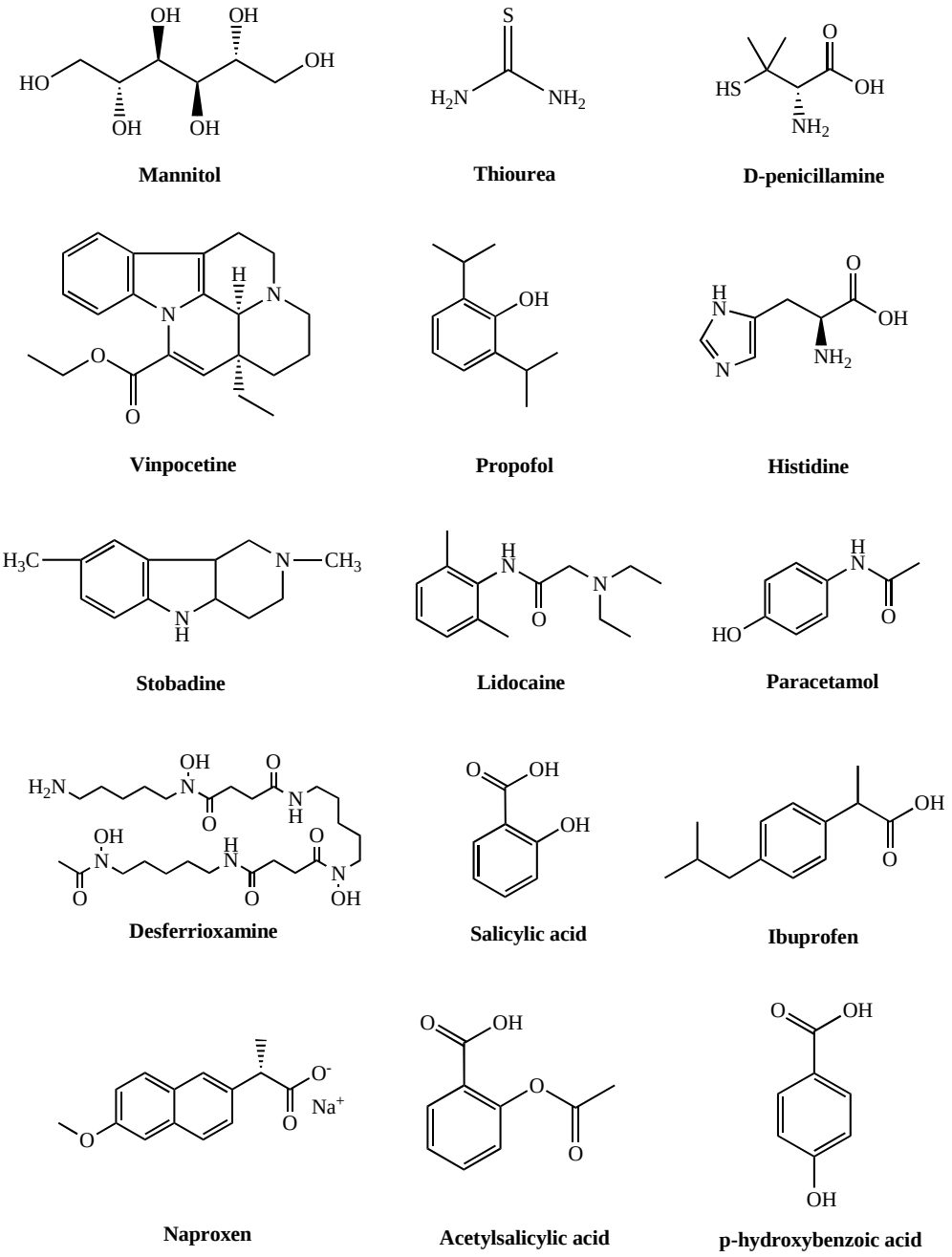


Fig. (2). Chemical structure of antioxidants protecting HA against oxidative damage.

depolymerization indicates an influence on the formation of ˙OH or on the radical itself [42].

These studies reveal the preventive or decreasing effect of a wide range of compounds in ROS-mediated HA degradation, which may be a good reason for putting into practice the supplementation of HA clinical preparations in order to maintain its integrity, though only mannitol is being used in viscosurgical devices.

THERAPEUTIC USES

HA therapeutic uses are related to physicochemical properties and cell biological functions, which depend on HA MW. Table (**1**) includes a compilation of the overall therapeutic and potential uses of HA and its derivatives, mainly related to their antioxidant profile.

There are large HA polymers which are space-filling, anti-angiogenic, immunosuppressive, and which impede differentiation, possibly by suppressing cell-cell interactions, or ligand access to cell surface receptors. HA chains, which can reach 20 MDa in size, are involved in ovulation, embryogenesis, protection of epithelial layer integrity, wound repair, and regeneration. Smaller polysaccharide fragments are proinflammatory, immuno-stimulatory and angiogenic. They can also compete with larger HA polymers for receptors. Low MW polymers appear to function as endogenous “danger signals”, while even smaller fragments can amelio-

rate these effects. Tetrasaccharides, for example, are anti-apoptotic and inducers of heat shock proteins [4,52].

Modification of HA allows the regulation of its properties depending on its usage: isolation of a certain range of HA MW, depolymerization of HA to obtain oligosaccharides and linkage of HA molecules to make a sponge, sheet or gel of HA. All these modifications have the same aim: to broaden and improve the therapeutic field of HA (Table (**1**)).

Degradation and depolymerization processes of high-molar-mass HA affect primary chemical structures of HA component units by producing fragments containing double bonds, opened sugar rings, scission of glycosidic linkages, abstraction of protons and production of unsaturated HA fragments [2]. Polymer fragmentation by free radicals depends on radical flux and occurs in a site-selective way [53]. Tetra and hexasacharides are the predominant products of hyaluronidase mediated degradation. Enzymatic cleavage mechanisms are in general more sparing of the HA macromolecule than free radical degradation. All these molecules would be recognized and an immune reaction might be triggered by such modified biomaterials [2,4]. Inhibition of HA degradation therefore may be crucial in reducing disease progression with an important therapeutic potential [4].

HA fragmentation mediated by free radicals could be a suitable methodology. It has been demonstrated in a redox system containing ascorbic acid and transition metal ions (Fe^{2+} and Cu^{2+}) that the reactive species generated were able to degrade high-molar mass HA [54]. The biogenic nature of this system would provide its application in biomaterials.

In addition, the several biological functions of HA correspond to the existence of different types of HA-binding proteins (hyaladherins). The most important is the main HA receptor, CD44. CD44 has diverse functions including not only the organization and metabolism of ECM, but it also engages the cytoskeleton and co-ordinates signaling events to enable cell response to changes in the environment [4].

HA Antioxidant Capacity Protecting Against Cellular Damage

Since cells within tissues are embedded in the ECM, it is of interest to know whether its components possess properties capable of modulating ROS. HA inhibits lipid peroxidation caused by oxidative stress and thereby decreases inflammatory reactions mediated by oxidants [55-57]. Anti-inflammatory effects after administration of different types of HA solutions into arthritic joints have also been reported, probably due to restoration of elastoviscosity of the synovial fluid [58]. However, some authors point out the protective effect of this biopolymer through neutralization of ROS generated during inflammation-driven oxidative burst by leukocytes and macrophages [59]. The suppression of inflammatory cytokine activity within the joint might be one important mechanism of the clinical action of intraarticular injection of HA in the treatment of OA [60].

In fact, the biological function of HA providing protection against cellular damage caused by radicals has been shown, both *in vitro* and *in vivo* [21,54,56,57], through its capacity to chelate transition metals [21,61]. Interleukin-1 (IL-1) induced oxidative stress and $O_2^{\cdot -}$ was also reduced by HA in bovine chondrocytes in a dose-dependent manner [62]. HA reduced cell damage induced by ·OH in a MW- and dose-dependent manner in avian embryonic fibroblasts [31] and in articular chondrocytes by oxygen-derived free radicals [32].

Zhao *et al*. [63] showed the protective effect of HA on oxidative DNA damage. Two possible mechanisms may account for the observed protective effect. One mechanism may be associated to the ability of HA to chelate ions [21]. Another mechanism may involve direct scavenging by HA of ROS, particularly the reactive products of Fenton's reaction, such as ·OH. The observed *in vitro* fragmentation of HA by H_2O_2 in the presence of $FeSO_4$ in tissue culture media is consistent with the second mechanism. In effect, the radical scavenging activity by HA results in its breakdown and

Table 1. Therapeutic and Potential Uses of HA and its Derivatives

Type of Compound	Therapeutic and Potential Uses
Lower MW HA (<0.5 MDa)	Angiogenesis regulation [98-101], inhibition of their function as danger signals [4,52], immune-stimulation and proinflammatory-effect inhibition [70, 80, 83], ciliary beat frequency stimulatory in lung disease [91].
Medium MW HA (0.5-1 MDa)	Viscosupplementation [58,72].
Higher MW HA (>1MDa)	Anti-angiogenic [98], antiinflammatory [82,96], cartilage protector [73-77], cell differentiation, cell migration, epithelial cell layer protector, immunosuppresive, regeneration, space-filling, stimulated HA synthesis [71], viscosupplementation [58,72], wound repair.
HA + Cations	Viscosupplementation [21,34-38].
Hylan	Viscosupplementation [39,72].
Esterified HA	Wound healing [3,22,41].
HA + Carboxymethylcellulose	Wound healing [3,22].
HA + Hydroxypropylmethylcellulose	Ophthalmic viscosurgical device [40].
HA + Mannitol	Ophthalmic viscosurgical device [42-46].

depletes the pool of oxidants that otherwise would damage DNA [63].

It is known that stem cells are equipped with a variety of defense mechanisms, particularly to protect their DNA from oxidative damage [64]. The presence of CD44 on the surface of stem cells is a well recognized fact [65], together with a highly effective efflux pump that rapidly removes genotoxic agents from the cell [66], and possibly internalization of HA, which protects DNA from oxidants.

Role of HA on Inflammatory Mediators

The role of HA in inflammatory mediators, including cytokines, proteases and their inhibitors, and prostaglandins may consist of cartilage protection and related therapeutic uses [67].

In synovial fluid from knee osteoarthritic joints, HA concentration is lower than in synovial fluid from normal knee joints. Additionally, experiments using rabbit synovial cells showed that the proinflammatory cytokines IL-1 and tumor necrosis factor α (TNF-α) regulate the expression of HA synthase [68], as well as contributing to the fragmentation of HA under inflammatory conditions by ROS [69]. Cleavage of HA results in the generation of variably sized fragments that stimulate multiple angiogenic and inflammatory responses [70].

Exogenous HA may facilitate the production of newly synthesized HA. When synovial fibroblasts from OA knees were cultured with HA formulations of various MWs (340-4700 kDa), the amount of newly synthesized HA was both concentration- and MW-dependent. Higher MW stimulated the synthesis of HA more than lower MW formulations and an optimal concentration was noted for each MW [71].

On the other hand, Moreland [72], on his review about mechanisms of action of HA in OA, reported a compilation of *in vitro* studies which have shown that HA alters the profile of inflammatory mediators, in such a way that the balance between cell matrix synthesis and degradation is shifted away from degradation. The proinflammatory cytokine TNF-α and its receptor were not evident in canine atrophied articular cartilage treated with HA but were observed in untreated cartilage [73]. In the synovium of rabbits in the early development of OA, HA also reduced the expression of IL-1β and stromelysin (matrix metalloproteinase 3 (MMP-3)) [74], two mediators known to play a role in cartilage degradation. The cartilage protective effect of high MW HA has been demonstrated in bovine articular chondrocytes, through the stimulation of the production of the tissue inhibitor of metalloproteinases 1 (TIMP-1). Although HA also stimulated stromelysin activity in the same study, the increase was inconsistent and lower with high MW than low MW HA. Furthermore, the stromelysin/TIMP-1 ratio was reduced [75]. The plasminogen activator system, shown to be active in synovial fibroblasts of rheumatoid arthritis, is also influenced by HA, as has been reported in synovial fibroblasts from OA and rheumatoid arthritis patients [76,77]. HA reduced the secreted antigen and activity of urokinase plasminogen activator, as well as its receptor.

The effects of HA on nitric oxide (NO), well recognized for its role in inflammation, may be tissue specific. Production of NO from the meniscus and synovium of a rabbit OA model was significantly reduced by HA treatment [78,79]. Other experiments have shown that HA did not affect NO production from articular cartilage [78]. In hepatic cells, fragments of HA increased the expression of the inducible form of NO synthase, while high MW HA did not have effects on its expression [80].

HA participates actively in the regulation of inflammatory and apoptotic processes, inhibiting the nuclear factor κB (NF-κB) and caspase activation during oxidative stress [81].

Recently, Campo *et al*. [82] showed that HA was able to improve chondrocyte survival and reduce NO levels in a dose dependent manner, although at the limit of the threshold of significance. Furthermore, the higher HA concentration slightly reduced proinflammatory cytokines, induced nitric oxide syntethase (iNOS), MMPs and caspase-3, while the lower concentration failed. Therefore, the lower HA concentration reduced NO levels without any effect on iNOS mRNA expression and protein production. This paradox may be justified by the fact that HA possesses a free radical scavenger activity and, consequently, may also directly bind to NO at this low concentration. Recently Zhou *et al*. [60], reported the effect of HA on IL-1-induced chondrocyte apoptosis in a rat model of OA. They showed that the addition of HA to the medium was able in a dose-dependent way to reduce the impairment of the mitochondrial membrane potential and to restore mitochondrial ATP production, and suppress chondrocyte apoptosis.

New Therapies Associated to HA

It has been established that HA can be an important regulator of the inflammatory process. As we described above, high MW HA can participate in restraining inflammation, while low MW possesses proinflammatory effects and activates immune cells. Through interaction with surface receptors (CD44, RHAMM, TLR4), HA fragments stimulate immune cells and enhance cytokine and ROS production as well as other factors participating in inflammation [83]. Therapies which can control or regulate this degradation seem able to reduce these pathologies.

The therapeutic use of HA, either by its direct effect (scavenging) or by its indirect role through cellular mediator, is revealed mainly in OA due to its higher affection in populations [72]. However, there are other diseases and pathologies associated to oxidative stress and immune cellular response that affect HA. Some aspects of these interactions remain to be elucidated, so it is not easy to find the link between hyaluronan receptors or some inflammatory mediators with the presence of HA from a therapeutic point of view. It is well-known that CD44 is considered the main HA receptor and HA-CD44 interactions participate in a wide range of cellular functions. In addition, CD44 is transcriptionally upregulated by proinflammatory cytokines such as IL-1 and growth factors. Some therapeutic approaches are based on the administration of HA-oligomers which compete for endogenous polymeric HA-receptor interaction [1,4]. The therapeutic role of CD44 adhesion to HA has also been shown since it mediates in chondrocyte proliferation and function [84]. When expression of CD44 was suppressed in

bovine articular cartilage slices, a near-complete loss of proteoglican-rich matrix was observed [85]. A similar decrease was found when very small HA molecules were used to block the binding of HA to the CD44 receptor [86].

Oxidants have been reported to play a role in both asthma and other lung diseases [87]. Accumulation of HA fragments, potentially arising from radical-mediated reactions at the site of injury in a CD44-deficient mouse model of pulmonary fibrosis [88], provides evidence for damage to lung. HA depolymerization by radicals may play a role in airway obstruction through activation of tissue kallokrein and epidermal growth factor receptors [89]. Protection from lung damage by extracellular superoxide dismutase (EC-SOD), which is highly expressed in the lung, is suggested [90]. The therapeutic role may reside in EC-SOD inhibition of pulmonary inflammation, in part by preventing $O_2^{\cdot -}$-mediated fragmentation of HA to low MW fragments. A recent study [49], shows that EC-SOD directly binds to HA, inhibiting significantly its degradation. In addition, high MW HA is broken down by ROS to form low MW fragments, which can act as a signal to stimulate ciliary beat frequency [91].

A reduction in HA MW of inflamed tissue in periodontal disease [92] has also been reported, and it has been suggested that oxidative damage may contribute to this. The protective actions of the SOD-mimetics [93] support a role for oxidant formation.

The dysregulation of HA metabolism is a typical feature of diabetes complications, and increased glucose level is considered to be the main cause of this phenomenon. HA depolymerization due to the effect of free radicals and advanced glycation end products leads to vitreous body liquefaction, and may be the reason for proliferative retinopathy in diabetes. The enrichment of ECM with high MW HA under the action of a high glucose level has been demonstrated for vascular smooth muscle cells, skin fibroblasts, endothelial and mesangial cells [94]. This effect is considered to accelerate the development of atherosclerosis [95], stimulating the proliferation of vascular smooth muscle cells, and to promote the transformation of acute wounds into chronic ulcers, deepening the pathological state of dermal fibroblasts in diabetes. And, conversely, the accumulation of high MW HA on the surface of endothelial cells may have a positive value for glycocalyx integrity.

Stern *et al.* [52] established that wound healing is an example of the precise regulation required of HA fragmentation. HA appears to have important biological roles in skin wound healing, by virtue of its presence in high amounts in skin, especially in granulation tissue during the wound healing process. High MW HA gel decreases the number of monocytes and macrophages in the early inflammatory phase of healing, as Schimizzi *et al.* [96] have demonstrated in the treatment of postlaminectomy wounds.

The ability of HA to create and fill space by organizing and modifying the ECM is widely used for soft tissue augmentation to limit age-related and photoinduced skin aging, but it may also be used for correction of facial lipodystrophy and to prevent recurrence of hypertrophic scars or keloids.

Other Therapeutic Uses of HA

Its wide range of biological functions allows HA to perform other therapeutic uses. Several of the physicochemical properties described above constitute the essential requirements for developing other HA applications. For instance, the capacity of steric exclusion of other macromolecules is another attribute of the molecular meshwork generated by HA by attracting water up to 99% of its own weight. In this sense, HA is essential in matrices to expand extracellular space. Some studies indicate that the expanded ECM present in the developing heart requires HA for stabilization [97], where HA binding motif interactions with proteins are determinant in stabilizing HA-rich matrices.

HA is also an angiogenic factor, with two different roles as a function of its MW [98]. High MW HA has been implicated in the differentiation and migration of many cell types, in the attraction of progenitor cells to sites of differentiation and in the inhibition of blood-vessel invasion. Oligosaccharides composed of 3 to 10 disaccharide units are able to stimulate angiogenesis [99]. These HA oligosaccharides specifically act on endothelial cells *in vitro*, stimulating cell proliferation and migration, and therefore contributing to capillary sprouts. This effect is related to HA role in tumorigenesis and metastasis [98], where HA levels are found to be increased. In this sense, the control of these levels may be a therapeutic target to reduce or inhibit tumor proliferation and invasiveness by inhibiting neovascularization [100,101]. Slevin *et al.* [100] also highlight the possible role of the cascade of reactions triggered by the interaction of HA with its receptors CD44 and RHAMM in angiogenesis and tumor spreading, and the difficulties arising from this to find new therapeutic approaches until these interactions and the associated intracellular signaling mechanisms are elucidated.

In this sense, the regulation of HA synthases appears to be important in inflammation and cancer progression. It has been demonstrated that HAS isoforms show different HA size distribution, probably in order to perform specific biological purposes [102]. The inhibition of low MW HA synthesis may imply a reduction in proinflammatory effects, as well as the inhibition of neovascularization, diminishing tumour progression. The significant role of the family of HAS1 protein in multiple myeloma progression [103] has been described, suggesting that HAS1 regulation may be a good approach in the treatment of this disease. On the other hand, the transfecting inhibition of HAS in prostate tumour cells has shown a reduction in tumour growth kinetics [104]. A better understanding of HA and HASs may facilitate the design of novel therapeutic strategies to counter presumptive cancer- and inflammation-promoting effects.

CONCLUSIONS

Current research on the physiological and pathological role of HA is opening up an exciting area of experimental therapeutics. The physical properties of HA are used as the basis for solutions for viscosurgery and viscosupplementation. On the other hand, chemical properties, mainly relating to its antioxidant capacity, give HA its scavenger role, protecting other macromolecules and structures. Moreover, interactions of HA with its receptors constitute a wide range of

cellular signalings involved in several biological, pathological and therapeutic processes. Further understanding of its effects and HA-receptor interactions may imply potentially fruitful therapeutic approaches in diseases as diverse as OA, diabetes or lung disease.

ACKNOWLEDGEMENTS

We are grateful to Prof. James McCue for assistance in language editing.

ABBREVIATIONS

ECM	=	Extracellular matrix
EC-SOD	=	Extracellular superoxide dismutase
GAG	=	Glycosaminoglycan
HA	=	Hyaluronan
H_2O_2	=	Hydrogen peroxide
IL-1	=	Interleukin-1
iNOS	=	Induced nitric oxide syntethase
MMP	=	Matrix metalloproteinase
MW	=	Molecular weight
NF-κB	=	Nuclear factor κB
NO	=	Nitric oxide
$O_2^{\cdot-}$	=	Superoxide radical
OA	=	Osteoarthritis
OH	=	Hydroxyl radical
$ONOO^-$	=	Peroxynitrite
ROS	=	Reactive oxygen species
SOD	=	Superoxide dismutase
TIMP	=	Tissue inhibitor of metalloproteinases
TNF-α	=	Tumor necrosis factor α

REFERENCES

[1] Garg, H.G.; Hales, C.A. *Chemistry and Biology of Hyaluronan;* Elsevier Ltd, Amsterdam, **2004.**

[2] Stern, R.; Kogan, G.; Jedrzejas, M.J.; Šoltés, L. The many ways to cleave hyaluronan. *Biotechnol., Adv.*, **2007**, *25*, 537-57.

[3] Moseley, R.; Leaver, M.; Walker, M.; Waddington, R.J.; Parsons, D.; Chen, W.Y.J.; Embery, G. Comparison of the antioxidant properties of HYAFF®-11p75, AQUACEL® and hyaluronan towards reactive oxygen species *in vitro*. *Biomaterials*, **2002,** *23*, 2255-64.

[4] Girish, K.S.; Kemparaju, K. The magic glue hyaluronan and its eraser hyaluronidase: a biological overview. *Life Sci.*, **2007**, *80*, 1921-43.

[5] Almond, A.; DeAngelis, P.L.; Blundell, C.D. Hyaluronan: the local solution conformation determined by NMR and computer modeling is close to a contracted left-handed 4-fold helix. *J. Mol. Biol.*, **2006,** *358*, 1256-69.

[6] Gura, E.; Hückel, M.; Müller, P.J. Specific degradation of rheological hyaluronic acid and its properties. *Polym. Degrad. Stabil.*, **1997,** *59*, 297-302.

[7] Almond, A. Visions & Reflections (Minireview). Hyaluronan. *Cell. Mol. Life Sci.*, **2007**, *64*, 1591-6.

[8] Cowman, M.K.; Matsuoka, S. Experimental approaches to hyaluronan structure. *Carbohydr. Res.*, **2005**, *340*, 791-809.

[9] Lepperdinger, G.; Fehrer, C.; Reitinger, S. In *Chemistry and biology of hyaluronan;* Elsevier Ltd, Amsterdam, **2004**; pp. 71-82.

[10] Flugge, L.A.; Miller-Deist, L.A.; Petillo, P.A. Towards a molecular understanding of arthritis. *Chem. Biol.*, **1999**, *6*, R157-66.

[11] Yamawaki. H.; Hirohata, S.; Miyoshi, T.; Takahashi, K.; Ogawa, H.; Shinohata, R.; Demircan, K.; Kusachi, S.; Yamamoto, K.; Ninomiya, Y. Hyaluronan receptors involved in cytokine induction in monocytes. *Glycobiology*, **2009**, *19*, *83-92*.

[12] Halliwell, B.; Whiteman, M. Measuring reactive species and oxidative damage *in vivo* and in cell culture: how should you do it and what do the results mean? *Br. J. Pharmacol.*, **2004,** *142*, 231-55.

[13] Young, I.S.; Woodside, J.V. Antioxidants in health and disease. *J. Clin. Pathol.*, **2001,** *54*, 176-86.

[14] Šoltés, L.; Stankovská, M.; Kogan, G.; Gemeiner, P.; Stern, R. Contribution of oxidative-reductive reactions to high-molecular-weight hyaluronan catabolism. *Chem. Biodivers.*, **2005**, *2*, *1242-5*.

[15] Rees, M.D.; Hawkins, C.L.; Davies, M.J. Hypochlorite and superoxide radicals can act synergistically to induce fragmentation of hyaluronan and chondroitin sulphates. *Biochem. J.*, **2004**, *381*, 175-84.

[16] Bergendi, L.; Beneš, L.; Ďuračková, Z.; Ferenčik, M. Chemistry, physiology and pathology of free radicals. *Life Sci.,* **1999**, *65*, 1865-74.

[17] Schiller, J.; Fuchs, B.; Arnhold, J.; Arnold, K. Contribution of reactive oxygen species to cartilage degradation in rheumatic diseases: molecular pathways, diagnosis and potential therapeutic strategies. *Curr. Med. Chem.*, **2003**, *10*, 2123-45.

[18] Šoltés, L.; Valachová, K.; Mendichi, R.; Kogan, G.; Arnhold, J.; Gemeiner, P. Solution properties of high-molar-mass hyaluronans: the biopolymer degradation by ascorbate. *Carbohydr. Res.*, **2007**, *342*, 1071-7.

[19] Halliwell, B.; Gutteridge, J.M.C. In: *Free Radicals in Biology and Medicine*; Oxford University Press: UK, **1999**; p. 106.

[20] Saari, H.; Konttinen, Y.T.; Friman, C.; Sorsa, T. Differential effects of reactive oxygen species on native synovial fluid and purified human umbilical cord hyaluronate. *Inflammation*, **1993**, *17*, 403-15.

[21] Balogh, G.T.; Illés, J.; Székely, Z.; Forrai, E.; Gere, A. Effect of different metal ions on the oxidative damage and antioxidant capacity of hyaluronic acid. *Arch. Biochem. Biophys.*, **2003**, *410*, 76-82.

[22] Moseley, R.; Walker, M.; Waddington, R.J.; Chen, W.Y.J. Comparison of the antioxidant properties of wound dressing materials–carboxymethylcellulose, hyaluronan benzyl ester and hyaluronan, towards polymorphonuclear leukocyte-derived reactive oxygen species. *Biomaterials*, **2003**, *24*, 1549-57.

[23] Trommer, H.; Wartewig, S.; Böttcher, R.; Pöppl, A.; Hoentsch, J.; Ozegowski, J.H.; Neubert, R.H.H. The effects of hyaluronan and its fragments on lipid models exposed to UV irradiation. *Int. J. Pharm.*, **2003**, *254*, 223-34.

[24] Halliwell, B. Superoxide-dependent formation of hydroxyl radicals in the presence of iron salts. Its role in degradation of hyaluronic acid by a superoxide-generating system. *FEBS Lett.*, **1978**, *96*, 238-42.

[25] Paimela, L.; Heiskanen, A.; Kurki, P.; Helve, T.; Leirisalo-Repo, M. Serum hyaluronate level as a predictor of radiologic progression in early rheumatoid arthritis. *Arthritis Rheum.*, **1991**, *34*, 815-21.

[26] Laurent, T.C.; Laurent, U.B.; Fraser, J.R. Serum hyaluronan as a disease marker. *Ann. Med.*, **1996**, *28*, 241-53.

[27] Moseley, R.; Waddington, R.J.; Embery, G. Degradation of glycosaminoglycans by reactive oxygen species derived from stimulated polymorphonuclear leukocytes. *Biochem. Biophys. Acta.*, **1997**, *1362*, 221-31.

[28] Tishler, M.; Yaron, I.; Shirazi, I.; Yaron, M. Salivary and serum hyaluronic acid concentrations in patients with Sjögren's syndrome. *Ann. Rheum. Dis.*, **1998**, *57*, 506-8.

[29] McHutchison; J.G.; Blatt, L.M.; de Medina, M.; Craig, J.R.; Conrad, A.; Schiff, E.R.; Tong, M.J. Measurement of serum hyaluronic acid in patients with chronic hepatitis C and its relationship to liver histology. Consensus Interferon Study Group. *J. Gastroenterol. Hepatol.*, **2000**, *15*, 945-51.

[30] Woollard, A.C.S.; Wolff, S.P.; Bascal, Z.A. Antioxidant characteristics of some potential anticataract agents. Studies of aspirin, paracetamol, and bendazac provide support for an oxidative component of cataract. *Free Radic. Biol. Med.*, **1990**, *9*, 299-305.

[31] Presti, D.; Scott, J.E. Hyaluronan-mediated protective effect against cell damage caused by enzymatically produced hydroxyl radicals is dependent on hyaluronan molecular mass. *Cell Biochem. Funct.*, **1994**, *12*, 281-288.

[32] Kvam, B.J.; Fragonas, E.; Degrassi, A.; Kvam, C.; Matulova, M.; Pollesello, P.; Zanetti, F.; Vittur, F. Oxygen-derived free radical (ODFR) action on hyaluronan (HA), on two HA ester derivatives, and on the metabolism of articular chondrocytes. *Exp. Cell Res.*, **1995**, *218*, 79-86.

[33] Cortivo, R.; Brun, P.; Cardarelli, L.; O'Regan, M.; Radice, M.; Abatangelo, G. Antioxidant effects of hyaluronan and its α-methyl-prednisolone derivative in chondrocyte and cartilage cultures. *Semin. Arthritis Rheum.*, **1996**, *26*, 492-501.

[34] Ialenti, A.; Di Rosa, M. Hyaluronic acid modulates acute and chronic inflammation. *Agents Actions*, **1994**, *43*, 44-7.

[35] Venge, P.; Pedersen, B.; Håkansson, L.; Hällgren, R.; Lindblad, G.; Dahl, R. Subcutaneous administration of hyaluronan reduces the number of infectious exacerbations in patients with chronic bronchitis. *Am. J. Respir. Crit. Care Med.*, **1996**, *153*, 312-6.

[36] Sakurai, K.; Andoh, M.; Yamada, M.; Kodera, Y.; Nishimura, H.; Hiroto, M.; Matsushima, A.; Aoyama, M.; Yamamoto, H.; Inada, Y. Suppression of ischemic edema in mice by manganese-hyaluronate conjugate. *Jpn. J. Pharmacol.*, **1997**, *74*, 117-20.

[37] Zeng, C.; Toole, B.P.; Kinney, S.D.; Kuo, J.W.; Stamenkovic, I. Inhibition of tumor growth *in vivo* by hyaluronan oligomers. *Int. J. Cancer*, **1998**, *77*, 396-401.

[38] Dougados, M. Sodium hyaluronate therapy in osteoarthritis: arguments for a potential beneficial structural effect. *Semin. Arthritis Rheum.*, **2000**, *30*, 19-25.

[39] Al-Assaf, S.; Phillips, G.O.; Deeble, D.J.; Parsons, B.; Starnes, H.; Von Sonntag, C. The enhanced stability of the cross-linked hylan structure to hydroxyl radicals compared with the uncrosslinked hyaluronan. *Radic. Phys. Chem.*, **1995**, *46*, 207-17.

[40] Maugeri, F.; Maltese, A.; Ward, K.W.; Bucolo, C. Hydroxyl radical scavenging activity of a new ophthalmic viscosurgical device. *Curr. Eye Res.*, **2007**, *32*, 105-11.

[41] Halliwell, B. Antioxidants in human health and disease. *Annu. Rev. Nutr.*, **1996**, *16*, 33-50.

[42] Lindvall, S.; Rydell, G. Influence of various compounds on the degradation of hyaluronic acid by a myeloperoxidase system. *Chem. Biol. Interact.*, **1994**, *90*, 1-12.

[43] Li, M.; Rosenfeld, L.; Vilar, R.E.; Cowman, M.K. Degradation of hyaluronan by peroxynitrite. *Arch. Biochem. Biophys.*, **1997**, *341*, 245-50.

[44] Orviský, E.; Šoltés, L.; Stančíková, M. High-molecular-weight hyaluronan – a valuable tool in testing the antioxidative activity of amphiphilic drugs stobadine and vinpocetine. *J. Pharm. Biomed. Anal.*, **1997**, *16*, 419-24.

[45] Belda, J. I.; Artola, A.; García-Manzanares, M. D.; Ferrer, C.; Haroun, H. E.; Hassanein, A.; Baeyens, V.; Muñoz, G.; Alió, J. L. Hyaluronic acid combined with mannitol to improve protection against free-radical endothelial damage: experimental model. *J. Cataract. Refract. Surg.*, **2005**, *31*, 1213-8.

[46] Mendoza, G.; Álvarez, A.I.; Pulido, M.M.; Molina, A.J.; Merino, G.; Real, R.; Fernandes, P.; Prieto, J.G. Inhibitory effects of different antioxidants on hyaluronan depolymerization. *Carbohydr. Res.*, **2007**, *342*, 96-102.

[47] Kvam, C.; Granese, D.; Flaibani, A.; Pollesello, P.; Paoletti, S. Hyaluronan can be protected from free-radical depolymerisation by 2,6-diisopropylphenol, a novel radical scavenger. *Biochem. Biophys. Res. Commun.*, **1993**, *193*, 927-33.

[48] Valachová, K.; Hrabarova, E.; Gemeiner, P.; Šoltés, L. Study of pro- and anti-oxidative properties of D-penicillamine in a system comprising high-molar-mass hyaluronan, ascorbate, and cupric ions. *Neuro. Endocrinol. Lett.*, **2008**, *29*, 697-701.

[49] Gao, F.; Koenitzer, J.R.; Tobolewski, J.M.; Jiang, D.; Liang, J.; Noble, P.W.; Oury, T.D. Extracellular superoxide dismutase inhibits inflammation by preventing oxidative fragmentation of hyaluronan. *J. Biol. Chem.*, **2008**, *283*, 6058-66.

[50] Šoltés, L.; Stankovská, M.; Kogan, G.; Mendichi, R.; Volpi, N.; Sasinková, V.; Gemeiner, P. Degradation of high-molar-mass hyaluronan by an oxidative system comprising ascorbate, Cu(II), and hydrogen peroxide: inhibitory action of antiinflammatory drugs--naproxen and acetylsalicylic acid. *J. Pharm. Biomed. Anal.*, **2007**, *44*, 1056-63.

[51] Šoltés, L.; Lath, D.; Mendichi, R.; Bystrický, P. Radical degradation of high molecular weight hyaluronan: inhibition of the reaction by ibuprofen enantiomers. *Methods Find Exp. Clin. Pharmacol.*, **2001**, *23*, 65-71.

[52] Stern, R.; Asari, A.A.; Sugahara, K.N. Hyaluronan fragments: an information-rich system. *Eur. J. Cell Biol.*, **2006**, *85*, 699-715.

[53] Kennett, E.C.; Davies, M.J. Glycosaminoglycans are fragmented by hydroxyl, carbonate, and nitrogen dioxide radicals in a site-selective manner: implications for peroxynitrite-mediated damage at sites of inflammation. *Free Radic. Biol. Med.*, **2009,** *47, 389-400*.

[54] Šoltés, L.; Valachová, K.; Mendichi, R.; Kogan, G.; Arnhold, J.; Gemeiner, P. Solution properties of high-molar-mass hyaluronans: the biopolymer degradation by ascorbate. *Carbohydr. Res.*, **2007**, *342, 1071–7*.

[55] Albertini, R.; Passi, A.; Abuja, P.M.; De Luca, G. The effect of glycosaminoglycans and proteoglycans on lipid peroxidation. *Int. J. Mol. Med.*, **2000**, *6*, 126-36.

[56] Campo, G.M.; Avenoso, A.; Campo, S.; Ferlazzo, A.M.; Altavilla, A.; Calatroni, A. Efficacy of treatment with glycosaminoglycans on experimental collagen-induced arthritis in rats. *Arthritis Res. Ther.*, **2003**, *5*, R122-31.

[57] Campo, G.M.; Avenoso, A.; Campo, S.; Ferlazzo, A.M.; Altavilla, D.; Micali, C.; Calatroni, A. Aromatic trap analysis of free radicals production in experimental collagen-induced arthritis in the rat: protective effect of glycosaminoglycans treatment. *Free Radic. Res.*, **2003**, *37*, 257-68.

[58] Balazs, E.A. Viscosupplementation for treatment of osteoarthritis: from initial discovery to current status and results. *Surg. Technol. Int.*, **2004**, *12*, 278-89.

[59] Halicka, H.D.; Mitlitski, V.; Heeter, J.; Balazs, E.A.; Darzynkiewicz, Z. Attenuation of the oxidative burst-induced DNA damage in human leukocytes by hyaluronan. *Int. J. Mol. Med.*, **2009**, *23, 695-9*.

[60] Zhou, P.H.; Liu, S.Q.; Peng, H. The effect of hyaluronic acid on IL-1β-induced chondrocyte apoptosis in a rat model of osteoarthritis. *J. Orthop. Res.*, **2008**, *26*, 1643-8.

[61] Campo, G.M.; D'Ascola, A.; Avenoso, A.; Campo, S.; Ferlazzo, A.M.; Micali, C.; Calatroni, A. Glycosaminoglycans reduce oxidative damage induced by copper (Cu^{2+}), iron (Fe^{2+}) and hydrogen peroxide (H_2O_2) in human fibroblast cultures. *Glycoconjug. J.*, **2004**, *20*, 133-41.

[62] Fukuda, K.; Oh, M.; Asada, S.; Hara, F.; Matsukawa, M.; Otani, K.; Hamanishi, C. Sodium hyaluronate inhibits interleukin-1-evoked reactive oxygen species of bovine articular chondrocytes. *Osteoarthritis Cartilage*, **2001**, *9*, 390-2.

[63] Zhao, H.; Tanaka, T.; Mitlitski, V.; Heeter, J.; Balazs, E.A.; Darzynkiewicz, Z. Protective effect of hyaluronate on oxidative DNA damage in WI-38 and A549 cells. *Int. J. Oncol.*, **2008**, *32*, 1159-67.

[64] Stambrook, P.J. An ageing question: do embryonic stem cells protect their genomes? *Mech. Ageing Dev.*, **2007**, *128*, 31-5.

[65] Sales, K.M.; Winslet, M.C.; Seifalian, A. Stem cells and cancer: an overview. *Stem Cell Rev.*, **2007**, *3*, 249-55.

[66] Reeijmakers, M.H.G.P. ATP-binding-cassette transporters in hematopoietic stem cells and their utility as therapeutic targets in acute and chronic myeloid leukemia. *Leukemia*, **2007**, *21*, 2094-102.

[67] Kogan, G.; Šoltés, L.; Stern, R.; Gemeiner, P. Hyaluronic acid: a natural biopolymer with a broad range of biomedical and industrial applications. *Biotechnol. Lett.*, **2007**, *29, 17-25*.

[68] Tanimoto, K.; Ohno, S.; Fujimoto, K.; Honda, K.; Ijuin, C.; Tanaka, N.; Doi, T.; Nakahara, M.; Tanne, K. Proinflammatory cytokines regulate the gene expression of hyaluronic acid synthetase in cultured rabbit synovial membrane cells. *Connect Tissue Res.*, **2001**, *42*, 187-95.

[69] Saari, H. Oxygen derived free radicals and synovial fluid hyaluronate. *Ann. Rheum. Dis.*, **1991**, *50, 389-92*.

[70] de la Motte, C.; Nigro, J.; Vasanji, A.; Rho, H.; Kessler, S.; Bandyopadhyay, S.; Danese, S.; Fiocchi, C.; Stern, R. Platelet-derived hyaluronidase 2 cleaves hyaluronan into fragments that trigger monocyte-mediated production of proinflammatory cytokines. *Am. J. Pathol.*, **2009**, *174, 2254-64.*

[71] Smith, M.M.; Ghosh, P. The synthesis of hyaluronic acid by human synovial fibroblasts is influenced by the nature of the hyaluronate in the extracellular environment. *Rheumatol. Int.*, **1987**, *7*, 113-22.

[72] Moreland, L.W. Intra-articular hyaluronan (hyaluronic acid) and hylans for the treatment of osteoarthritis: mechanisms of action. *Arthritis Res. Ther.*, **2003**, *5*, 54-67.

[73] Comer, J.S.; Kincaid, S.A.; Baird, A.N.; Kammermann, J.R.; Hanson, R.R.; Ogawa, Y. Immunolocalization of stromelysin, tumor necrosis factor (TNF) alpha, and TNF receptors in atrophied canine articular cartilage treated with hyaluronic acid and transforming growth factor beta. *Am. J. Vet. Res.*, **1996**, *57*, 1488-96.

[74] Takahashi, K.; Goomer, R.S.; Harwood, F.; Kubo, T.; Hirasawa, Y.; Amiel, D. The effects of hyaluronan on matrix metalloproteinase-3 (MMP-3), interleukin-1beta (IL-1beta), and tissue inhibitor of metalloproteinase-1 (TIMP-1) gene expression during the development of osteoarthritis. *Osteoarthritis Cartilage*, **1999**, *7*, 182-90.

[75] Yasui, T.; Akatsuka, M.; Tobetto, K.; Umemoto, J.; Ando, T.; Yamashita, K.; Hayakawa, T. Effects of hyaluronan on the production of stromelysin and tissue inhibitor of metalloproteinase-1 (TIMP-1) in bovine articular chondrocytes. *Biomed. Res.*, **1992**, *13*, 343-8.

[76] Nonaka, T.; Kikuchi, H.; Ikeda, T.; Okamoto, Y.; Hamanishi, C.; Tanaka, S. Hyaluronic acid inhibits the expression of u-PA, PAI-1, and u-PAR in human synovial fibroblasts of osteoarthritis and rheumatoid arthritis. *J. Rheumatol.*, **2000**, *27*, 997-1004.

[77] Nonaka, T.; Kikuchi, H.; Shimada, W.; Itagene, H.; Ikeda, T.; Hamanishi, C.; Tanaka, S. Effects of hyaluronic acid on fibrinolytic factors in the synovial fluid (*in vivo*). *Pathophysiology*, **1999**, *6*, 41-4.

[78] Takahashi, K.; Hashimoto, S.; Kubo, T.; Hirasawa, Y.; Lotz, M.; Amiel, D. Hyaluronan suppressed nitric oxide production in the meniscus and synovium of rabbit osteoarthritis model. *J. Orthop. Res.*, **2001**, *19*, 500-3.

[79] Díaz-Gallego, L.; Prieto, J.G.; Coronel, P.; Gamazo, L.E.; Gimeno, M.; Álvarez, A.I. Apoptosis and nitric oxide in an experimental model of osteoarthritis in rabbit after hyaluronic acid treatment. *J. Orthop. Res.*, **2005**, *23*, 1370-6.

[80] Rockey, D.C.; Chung, J.J.; McKee, C.M.; Noble, P.W. Stimulation of inducible nitric oxide synthase in rat liver by hyaluronan fragments. *Hepatology.*, **1998**, *27*, 86-92.

[81] Yasuda, T. Hyaluronan inhibits cytokine production by lypopolysaccharide-stimulated U937 macrophages through down-regulation of NF-kappaB via ICAM-1. *Inflamm. Res.*, **2007**, *56*, 246-53.

[82] Campo, G.M.; Avenoso, A.; Campo, S.; D'Ascola, A.; Traina, P.; Samà, D.; Calatroni, A. Glycosaminoglycans modulate inflammation and apoptosis in LPS-treated chondrocytes. *J. Cell Biochem.*, **2009**, *106*, 83-9.

[83] Casalino-Matsuda, S.M.; Monzon, M.E.; Day, A.J.; Forteza, R.M. Hyaluronan fragments/CD44 mediate oxidative stress-induced MUC5B up-regulation in airway epithelium. *Am. J. Respir. Cell Mol. Biol.*, **2009**, *40*, 277-85.

[84] Ishida, O.; Tanaka, Y.; Morimoto, I.; Takigawa, M.; Eto, S. Chondrocytes are regulated by cellular adhesion through CD44 and hyaluronic acid pathway. *J. Bone Miner. Res.*, **1997**, *12*, 1657-63.

[85] Chow, G.; Nietfeld, J.J.; Knudson, C.B.; Knudson, W. Antisense inhibition of chondrocyte CD44 expression leading to cartilage chondrolysis. *Arthritis Rheum.*, **1998**, *41*, 1411-9.

[86] Knudson, W.; Loeser, R.F. CD44 and integrin matrix receptors participate in cartilage homeostasis. *Cell Mol. Life Sci.*, **2002**, *59*, 36-44.

[87] Andreadis, A.A.; Hazen, S.L.; Comhair, S.A.A.; Erzurum, S.C. Oxidative and nitrosative events in asthma. *Free Radic. Biol. Med.*, **2003**, *35*, 213-25.

[88] Teder, P.; Vandivier, R.W.; Jiang, D.H.; Liang, J.R.; Cohn, L.; Pure, E.; Henson, P.M.; Noble, P.W. Resolution of lung inflammation by CD44. *Science*, **2002**, *296*, 155-8.

[89] Casalino-Matsuda, S.M.; Monzon, M.E.; Conner, G.E.; Salathe, M.; Forteza, R.M. Role of hyaluronan and reactive oxygen species in tissue kallikrein-mediated epidermal growth factor receptor activation in human airways. *J. Biol. Chem.*, **2004**, *279*, 21606-16.

[90] Fattman, C.L.; Schaefer, L.M.; Oury, T.D. Extracellular superoxide dismutase in biology and medicine. *Free Radic. Biol. Med.*, **2003**, *35*, 236-56.

[91] Manzanares, D.; Monzon, M.E.; Savani, R.C.; Salathe, M. Apical oxidative hyaluronan degradation stimulates airway ciliary beating via RHAMM and RON. *Am. J. Respir. Cell Mol. Biol.*, **2007**, *37*, 160-8.

[92] Bartold, P.M.; Page, R.C. The effect of chronic inflammation on gingival connective. *J. Oral Pathol.*, **1986**, *15*, 367-74.

[93] Di Paola, R.; Mazzon, E.; Rotondo, F.; Dattola, F.; Britti, D.; De Majo, M.; Genovese, T.; Cuzzocrea, S. Reduced development of experimental periodontitis by treatment with M40403, a superoxide dismutase mimetic. *Eur. J. Pharmacol.*, **2005**, *516*, 151-7.

[94] Yevdokimova, N.Y. Elevated level of ambient glucose stimulates the synthesis of high-molecular-weight hyaluronic acid by human mesangial cells. The involvement of transforming growth factor beta1 and its activation by thrombospondin. *Acta. Biochim. Pol.*, **2006**, *53*, 383-93.

[95] Bot, P.T.; Hoefer, I.E.; Piek, J.J.; Pasterkamp, G. Hyaluronic acid: targeting immune modulatory components of the extracellular matrix in atherosclerosis. *Curr. Med. Chem.*, **2008**, *15*, 786-91.

[96] Schimizzi, A.L.; Massie, J.B.; Murphy, M.; Perry, A.; Kim, C.W.; Garfin, S.R.; Akeson, W.H. High-molecular-weight hyaluronan inhibits macrophage proliferation and cytokine release in the early wound of a preclinical postlaminectomy rat model. *Spine J.*, **2006**, *6*, 550-6.

[97] Mjaatvedt, C.H.; Yamamura, H.; Capehart, A.A.; Turner, D.; Markwald, R.R. The Cspg2 gene, disrupted in the hdf mutant, is required for right cardiac chamber and endocardial cushion formation. *Dev. Biol.*, **1998**, *202*, 56-66.

[98] Rooney, P.; Kumar, S.; Ponting, J.; Wang, M. The role of hyaluronan in tumour neovascularization. *Int. J. Cancer*, **1995**, *60*, 632-6.

[99] Sattar, A.; Rooney, P.; Kumar, S.; Pye, D.; West, D.C.; Scott, I.; Ledger, P. Application of angiogenic oligosaccharides of hyaluronan increase blood-vessel numbers in skin. *J. Invest. Dermatol.*, **1994**, *103*, 576-9.

[100] Slevin, M.; Krupinski, J.; Gaffney, J.; Matou, S.; West, D.; Delisser, H.; Savani, R.C.; Kumar, S. HA-mediated angiogenesis in vascular disease: uncovering RHAMM and CD44 receptor signaling pathways. *Matrix Biol.*, **2007**, *26*, 58-68.

[101] Heldin, P.; Karousou, E.; Bernert, B.; Porsch, H.; Nishitsuka, K.; Skandalis, S.S. Importance of HA-CD44 interactions in inflammation and tumorigenesis. *Connect. Tissue Res.*, **2008**, *49*, 215-8.

[102] Weigel P.H. . In *Chemistry and Biology of Hyaluronan;* Elsevier Ltd, Amsterdam, **2004**; pp. 553-567.

[103] Adamia, S.; Reiman, T.; Crainie, M.; Mant, M.J.; Belch, A.R.; Pilarski, L.M. Intronic splicing of hyaluronan synthase 1 (HAS1): a biologically relevant indicator of poor outcome in multiple myeloma. *Blood*, **2005**, *105, 4836-44.*

[104] Simpson, M.A.; Wilson, C.M.; McCarthy, J.B. Inhibition of prostate tumor cell hyaluronan synthesis impairs subcutaneous growth and vascularization in immunocompromised mice. *Am. J. Pathol.*, **2002**, *161, 849-57.*

Received: 04 August, 2009 Revised: 28 October, 2009 Accepted: 28 October, 2009