



HYALURONAN AND ITS APPLICATIONS – A REVIEW

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ABSTRACT

Hyaluronan (or Hylauronic acid - HA) a polysaccharide found in all tissues and body fluids of vertebrates as well as in some bacteria. Hyaluronan with the structure D-glucuronic acid (1-β-3) N-Acetyl -D- glucosamine (1-β4)_n is a linear polymer built from repeating disaccharide units. It is available in various forms like mouthwashes, sprays and Gel form is the most common available form of hyaluronic acid. It is available in various concentrations 0.2% and 0.8%. Hyaluronan enhances tissue regeneration, stimulates osteoinduction, is involved in the process of osseointegration and plays an important role in the early stages of wound healing, antiinflammatory properites. Hyaluronan has a multifunctional role in periodontics as an adjunct to scaling and root planing, topical application of sub gingival hyaluronic acid gel can be used as an antimicrobial agent, as abone regenerating agent in periodontal bony defects, in Guided bone regeneration., in Peri-implant maintenance of immediate function implant and as an autologous cell hyaluronic acid graft for gingival augmentation in mucogingival surgeries. Topical HA 0.2% or gel forms a protective coating around the oral cavity to shield exposed or sensitized nerve endings from overstimulation. Hyaluronic acid (as sodium hyaluronate coats the oral mucosa, enhances tissue hydration, and accelerates healing. Therefore this review focuses on the structure, properites and various clinical application s of hyaluronan.

KEYWORDS: Hyaluronan, Gengigel, periodontitis, Gingival Crevicular fluid.

Hyaluronan (or Hylauronic acid - HA) a polysaccharide found in all tissues and body fluids of vertebrates as well as in some bacteria. It is a multifunctional glycosaminoglycan (GAG) which forms an essential component of the extracellular matrix (ECM) in connective tissue and forms the basis of the pericellular matrix. In the Oral cavity, it is highly dense in the superficial layers of the buccal mucosa not only contributing to the stability and elasticity of the tissue but also an epithelial barrier effect.^[1]

CHEMICAL STRUCTURE

Hyaluronan with the structure D-glucuronic acid (1-β-3) N-Acetyl -D- glucosamine (1-β4)_n is a linear polymer built from repeating disaccharide units (Meyer et al).

According to Scott, the molecule is stabilized by the hydrogen bonds parallel with the chain.^[2]

It-is synthesized by 3 different but related hyaluronan synthases(HAS), HAS1, HAS2 and HAS3 which are enzymes with multiple transmembrane domains that

synthesize hyaluronan at the inner surface of the plasma membrane.^[3]

It has a high molecular weight between 10³ and 10⁴ kDa and an extended length of 2–25 μm. It is found highest in soft connective tissues in humans (umbilical cord, synovial fluid, skin and the vitreous body) and lowest concentration in the serum. Added to this it is also present in notable amounts in lung, kidney, brain and muscle and a very little in liver. Hyaluronan binds to many other extracellular matrix molecules, through cell surface receptors specifically to cell bodies, and has a unique mode of synthesis in which the molecule is immediately transported into the extracellular space upon formation.^[4]

METABOLISM

Major part of the metabolism of hyaluronan occurs in the liver. Tissues which are densely structured and with no lymphatic drainage, it is probably that most of the hyaluronic acid turn over occurs by metabolic degradation in situ.^[2]

In liver it is taken up by endocytosis, transported to lysosomes where it is degraded by the enzymes β - N - acetyl glucosaminidase, hyaluronidase and β glucuronidase. The degradation products are glucuronic acid and N- acetyl glucosamine, the monosaccharides are transferred to cytosol where the glucuronic acid is degraded via regular pathway. The N-acetyl glucosamine further undergoes phosphorylation and acetylation to form carbon dioxide and water and deamination before entering the glycolytic pathway.^[4]

PROPERTIES

Hyaluronan has multifaceted roles because of its physicochemical and biological properties. These biological roles range from developmental regulation through effects of cellular behaviour via control of the tissue macro and microenvironments to a purely structural function in the extracellular matrix, as well as through direct receptor mediated effects on gene expression. Amongst extracellular matrix molecules, it has unique viscoelastic and hygroscopic properties.^[1]

Hygroscopic property

HA is readily soluble in water, producing a gel that behaves by absorbing water as a lubricant, giving the hygroscopic and homeostatic properties. Because of its high solubility it is found to be problematic in the development of polymers for tissue engineering. HA when in contact even with a small amount of water undergoes liquefaction. But the same hygroscopic nature may be beneficial in some applications as in orthopaedic surgery. Alcohol esterification using benzyl alcohol is the usual method of stabilisation.^[5]

Viscoelastic property

HA exhibits a high resistance against water flow and can thus act in tissues as a barrier against rapid changes in water content. A very interesting feature of this property is that the penetration of viruses / bacteria is slowed down, is of paramount importance as in periodontal infections. Therefore as a viscoelastic substance it assists in periodontal regenerative procedures by maintaining spaces and protecting surfaces.

Hyaluronan solutions also exhibit a non-ideal osmotic pressure i.e the osmotic pressure increases exponentially rather than linearly with increasing concentration of hyaluronan. This property gives hyaluronan solutions osmotic buffering capacity, which could be useful in regulating the water content in a tissue.^[6]

FUNCTIONS

The well known function of hyaluronan which is reputed for several years, is to serve as a 'binding agent' between various connective tissue components. However, several studies suggested that it has various functions that include an important role in cell adhesion, migration and differentiation. Also to note is that the production of HA is also increased *in vitro* by the action of bacterial lipopolysaccharides (Bartold, 1991). Hyaluronan

enhances tissue regeneration (Nakamura, 1993), stimulates osteoinduction (Sakasi and Watanabe, 1995), is involved in the process of osseointegration (Klinger et al, 1998) and plays an important role in the early stages of wound healing (Weigel et al, 1986). It has adhesive properties and also possesses anti-inflammatory properties (Vangelisti et al, 1993; Pagnacco et al, 1997; Jentsch et al, 2003). It is shown to have both a bacteriostatic effect (Pirnazar et al, 1999) and antioxidant effect (Fukuda et al, 1999).^[7]

Permeability & Transport

By acting as a sieve HA allows small molecules move freely while larger particles are immobilised. Also it has been proposed that hyaluronan and other polysaccharides regulate transport of other macromolecules through the extracellular space.^[6]

Osteoinductive and Osteoconductive property

Hyaluronic acid also has bone induction characteristics with osteogenic substrates such as bone morphogenic protein (BMP-2), calcitonine gene- related peptide (CgRPa) and osteopontin. It accelerates the bone regeneration by means of proliferation, chemotaxis and successive differentiation of mesenchymal cells.^[8] Recent studies also demonstrated that HA aids in the repair process of both soft and hard tissue.^[9] Hence it has been suggested that HA could be used for bone regeneration in periodontal/peri-implant diseases.^[10]

Antiproliferative effect

High molecular weight HA reduces cell proliferation in fibroblasts and lymphocytes as well as in epithelial cells which abates the inflammatory process. This property is useful in improving the periodontal lesion as in patients with chronic periodontitis.^[11] **Anti- hyaluronidase effect** Hyaluronidase is a bacterial enzyme which plays an important role in 'plaque induced' diseases due to its ability to break down the proteoglycans (PG) and GAGs in the ground substance of connective tissue (ie. macroaggregates of hyaluronic acid and proteins), thereby enabling bacteria to invade even the deepest periodontal structures. As a result of its physiological macroaggregating activity high molecular weight hyaluronic acid can therefore perform an efficient anti-hyaluronidase action.^[12]

Anti – oedematous effect

Oedema basically consists of an accumulation of fluid in the intercellular spaces of connective tissue. In the proteoglycans, the macroaggregating effect of hyaluronic acid gives rise to "free water binding". Therefore capture of free water resulting from the formation of hydrogen bonds produces an anti oedematous effect helping in tailing the inflammation.^[12]

Bacteriostatic effect

Regardless of the concentration or molecular weight-HA has no bactericidal effect but it exhibits bacteriostatic effect. Significant bacteriostatic effects were observed

regardless of the concentration or molecular weight for *S. Aureus* and to a greater extent for *A. Actinomycetencomitans*. The most significant bacteriostatic effects on both these strains were observed with high concentrations of medium molecular weight Hyaluronic acid. It has least bacteriostatic effect on *S. Mutans* and *P. Gingivalis* strains.^[9]

Effect on angiogenesis

Low molecular weight HA has a marked angiogenic effect whereas, surprisingly, high molecular weight has the opposite effect.^[1]

Role in wound healing

High concentration of HA has been demonstrated in tissue repair. It is believed to play a role in wound healing by facilitating cell migration and differentiation during embryonic development and tissue repair. Numerous proteins have been shown to bind HA, including fibrinogen, fibrin, fibronectin and collagen. All these molecules help in wound healing and are also present even in foetal wound matrix.^[10] With the increased concentration of hyaluronan can modulate foetal wound healing by orchestrating healing through regeneration rather than scarring (**Longaker et al, 1991 and Adzick 1991**).^[13]

It stimulates the neutrophils and macrophages by enhancing their phagocytic activity, and further stimulates the release of chemotactic factors for the fibroblasts and helps in fibrin development. Moreover, it induces the fibroblasts proliferation and stimulates their metabolism during the granulation phase of the healing process, with a subsequent increase in collagenous fibers and in fundamental substance deposition.^[14]

Modulation of inflammation

An increased tissue accumulation of HA occurs in human and experimental inflammatory conditions such as in the case of sarcoidosis, inflammatory bowel disease, idiopathic pulmonary fibrosis and farmer's lung. There is an increase in the elimination of hyaluronan from the tissue compartments during the inflammation and there is an increased turnover rate of hyaluronan in the affected tissue. There is increase in the cytokine release from the inflammatory cells which influences the synthesis of hyaluronan.^[15]

Anti inflammatory effect

It is a glycosaminoglycan with anti inflammatory effect. Its anti-inflammatory effect may be due to the action of exogenous hyaluronan as a scavenger by draining prostaglandins, metalloproteinases and other bio-active molecules (**Laurent et al. 1995**).^[16]

CLINICAL APPLICATIONS

HA is a valued therapeutic agent in various fields like dermatology^[1], ophthalmology^[2], otolaryngology^[3], orthopedics^[9] and more recently in plastic surgery. There is documented toxic effects and no contraindications to

its use till date. Its activities against infection and inflammation together with its capacity to promote wound healing had extended its applications to include dentistry.^[2]

Hyaluronan has been identified in all periodontal tissues in varying quantities, being more prominent in the nonmineralized tissues compared to mineralized tissues. In addition, due to the high levels of hyaluronan in blood serum, it constantly present in gingival crevicular fluid (GCF) as a serum overload factor.^[1]

Medical and Surgical Applications

HA is used in ophthalmology for reducing intraocular pressure, carrier for topical antibiotics and in management of dry eye as in Sjogren's syndrome. In pulmonary medicine it is useful in maintaining the patency of airway.^[5] Since HA is an intrinsic component of articular cartilage, it is critical to joint development in the embryo and subsequent adult function, although it may not be the most important lubricant. Hence it is used in orthopaedics for symptomatic management of osteoarthritis.^[4,6] The most common use for HA in rejuvenative medicine is as a filler for wrinkles, cutaneous lines and breast prosthesis. As a diagnostic marker it is increased from 10-100 folds in cirrhosis of liver and in graft rejection cases.^[6,8]

CLINICAL APPLICATIONS IN DENTISTRY

Treatment of gingivitis

HA is a component of the connective tissue and was detected in the gingiva (**Bartold et al. 1981, Giannobile et al. 1993**). It is available in various forms like mouthwashes, sprays and Gel form is the most common available form of hyaluronic acid. It is available in various concentrations 0.2% and 0.8%. It has been studied as a metabolite or marker of inflammation in the gingival crevicular fluid (GCF) (**Embery et al. 1982, Last et al. 1985, Smith et al. 1996**) as well as a significant factor in growth and development and repair of tissues (**Pogrel et al. 1996**). High molecular weight HA stimulates early healing and reparative dentine formation (**Sasaki & Kawamata-Kido 1995**). The anti-inflammatory effect may be due to the action of exogenous hyaluronan as a scavenger by inhibiting prostaglandins, metalloproteinase and other bio-active molecules (**Laurent et al. 1995**). The anti oedematous effect may also be related to the osmotic activity. Studies have shown that hyaluronan containing gel has a beneficial effect during the therapy of plaque induced gingivitis.^[17] Studies indicated that there is significant therapeutic effect of hyaluronan in the treatment of gingivitis.^[18]

Treatment of chronic periodontitis

The local application of hyaluronan gel in conjunction with scaling and root planing have a beneficial effect on periodontal health in patients with chronic periodontitis. High molecular-weight HA gel reduces cell proliferation in gingival epithelial cells, lymphocytes and fibroblasts,

abates the inflammatory process, and improves periodontal lesions in patients with chronic periodontitis. Recent studies have shown that esterified hyaluronic acid can be used in the treatment of periodontal intra bony defects. It induces significant reduction in pocket depth and promotes gain in clinical attachment level.^[19,20]

Hyaluronan has a multifunctional role in periodontics

1. As an adjunct to scaling and root planing topical application of sub gingival hyaluronic acid gel can be used as an antimicrobial agent.
2. Bone regeneration in periodontal bony defects.
3. Guided bone regeneration.
4. Non surgical treatment of peri-implant pockets.
5. Peri-implant maintenance of immediate function implants
6. As autologous cell hyaluronic acid graft, gingival augmentation in mucogingival surgery.
7. In various regenerative procedures it acts as a carrier for newer molecules.
8. In tissue engineering research used as a biomaterial scaffold.

Treatment of recurrent aphthous ulcers (RAU)

RAUs is a common lesion which typically manifest as a small erosion of the epithelial layer of the non keratinized oral mucosa associated with significant pain that interferes with mastication, speech and deglutition.^[24]

Hyaluronan is an endogenous polysaccharide that is present in the basement membrane of wide variety of tissues including in the oral mucosa. HA is used singly as 0.2% gel or in combination with 3% diclofenac in 2.5%hyaluronan is an effective treatment for this common painful disorder.^[21]

It gives immediate relief from soreness after topical application. Hyaluronan 0.2% offers better advantage over steroids in that it is safe for all patients including infants and pregnant women.^[22]

Topical HA 0.2% or gel forms a protective coating around the oral cavity to shield exposed or sensitized nerve endings from overstimulation. Hyaluronic acid (as sodium hyaluronate coats the oral mucosa, enhances tissue hydration, and accelerates healing.^[23]

Regenerative therapy

Apatite coated HA is effective in promoting differentiation of osteoblast like cells and bone formation during the repair of bone defect in vitro. In vivo it is used in the treatment of various bone defects. HA primarily acts locally at the site to promote healing by stimulating callus formation. It shares bone induction characteristics with osteogenic substrate such as calcitonin and BMP. Recent studies have shown that it aids in the repair of both soft tissues and bone.^[24]

Management of oral lichen planus (OLP)

OLP is a chronic immune-inflammatory disease of the oral mucosa. The pathogenesis of OLP remains uncertain, but it is thought to arise from an immune response to a number of antigens within the oral epithelium. In the management of erosive OLP topical HA (0.2%) does appear to be of some benefit providing efficacy for up to 4 h after administration. To obtain a more significant clinical benefit frequent applications should be considered. Hence topical HA gel may be useful as an additional treatment options for OLP.^[25]

Protective Function

HA is also present in saliva and its levels vary depending on the type of saliva and the condition at the time of collection. The concentration of hyaluronan is highest in unstimulated whole saliva and lowest in stimulated parotid saliva. Hyaluronan contributes to the healing properties of the saliva, assisting in protecting the oral mucosa and adding to the lubricating properties of the saliva.^[13]

SAFETY

Hyaluronic acid is native to the body, it is non immunogenic, biocompatible with no evidence of cytotoxicity. Hyaluronan gel, injections or orally should not be administered in patients with allergies.^[26]

ADVERSE EFFECTS

The adverse effects of HA although not severe include bruising redness, pain, swelling, itching and tenderness at the injection site.^[1]

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