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HYALURONIC ACID IS ONE OF THE MOST HYDROPHILIC MOLECULES IN NATURE CAN BE DESCRIBED AS NATURE'S MOISTURIZER

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ABSTRACT

Hyaluronic acid (HA) is a high molecular weight biopolysacharide, discovered in 1934, by Karl Meyer and his assistant, John Palmer in the vitreous of bovine eyes. Hyaluronic acid is a naturally occurring biopolymer, which has important biological functions in bacteria and higher animals including humans. It is found in most connective tissues and is particularly concentrated in synovial fluid, the vitreous fluid of the eye, umbilical cords and chicken combs. It is naturally synthesized by a class of integral membrane proteins called hyaluronan synthases, and degraded by a family of enzymes called hyaluronidases. This review describes metabolisms, different physiological and pathological functions, basic pharmacological properties, and the clinical use of hyaluronic acid.

KEYWORDS: hyaluronic acid, metabolism, toxicity, glycosaminoglycan, biopolysacharide, aminosugar.

Figure-1: Karl Meyer; discoverer of HA.

INTRODUCTION

Hyaluronic acid [Formula: $(C_{14}H_{21}NO_{11})_n$; CAS: 9004-61-9] also called hyaluronan, is an anionic, nonsulfated glycosaminoglycan distributed widely throughout connective, epithelial, and neural tissues. It is unique among glycosaminoglycans in that it is nonsulfated, forms in the plasma membrane instead of the Golgi apparatus, and can be very large: human synovial HA averages about 7 million Da per molecule, or about 20,000 disaccharide monomers, while other sources mention 3–4 million Da.[1] Hyaluronic acid (HA) is a carbohydrate, more specifically a mucopolysaccharide, occurring naturally in all living organisms. It can be several thousands of sugars (carbohydrates) long. When not bound to other molecules, it binds to water giving it a stiff viscous quality similar to "Jello". The polysaccharide hyaluronan (HA) is a linear polyanion, with a poly repeating disaccharide structure [(1→3)-β-d-GlcNAc-(1→4)-β-d-GlcA-]. HA is found primarily in the extracellular matrix and pericellular matrix, but has

also been shown to occur intracellularly. The biological functions of HA include maintenance of the elastoviscosity of liquid connective tissues such as joint synovial and eye vitreous fluid, control of tissue hydration and water transport, supramolecular assembly of proteoglycans in the extracellular matrix, and numerous receptor mediated roles in cell detachment, mitosis, migration, tumor development and metastasis, and inflammation.[2] Its function in the body is, amongst other things, to bind water and to lubricate movable parts of the body, such as joints and muscles. Its consistency and tissue-friendliness allows it to be used in skin-care products as an excellent moisturizer. The unique viscoelastic nature of HA along with its biocompatibility and non-immunogenicity has led to its use in a number of clinical applications, including the supplementation of joint fluid in arthritis as a surgical aid in eye surgery, and to facilitate the healing and regeneration of surgical wounds. More recently, HA has been investigated as a drug delivery agent for various administration routes, including ophthalmic, nasal, pulmonary, parenteral and topical.[3]

Physicochemical and structural properties: Hyaluronan, an extracellular matrix component, is a high molecular weight glycosamino-glycan composed of disaccharide repeats of N-acetylglucosamine and glucuronic acid. This relatively simple structure is conserved throughout all mammals, suggesting that HA is a biomolecule of considerable importance. In the body, HA occurs in the salt form, hyaluronate, and is found in high concentrations in several soft connective tissues, including skin, umbilical cord, synovial fluid, and vitreous humor. Significant amounts of HA are also found in lung, kidney, brain, and muscle tissues.[4]

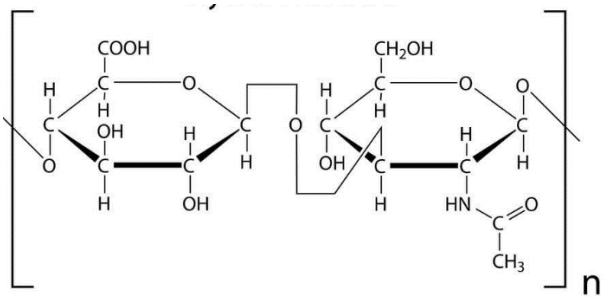

Figure-2: Hyaluronic acid.

Chemical structure: The uronic acid and aminosugar in the disaccharide are d-glucuronic acid and d-N-acetyl-glucosamine, and are linked together through alternating β-1,4 and β-1,3 glycosidic bonds (Figure-2). Both sugars are spatially related to glucose which in the β configuration allows all of its bulky groups (the hydroxyls [-OH], the carboxylate [-COOH] moiety and the anomeric carbon on the adjacent sugar) to be in sterically favorable equatorial positions while all of the small hydrogen atoms occupy the less sterically favourable axial positions. Thus, the structure of the disaccharide is energetically very stable.[5]

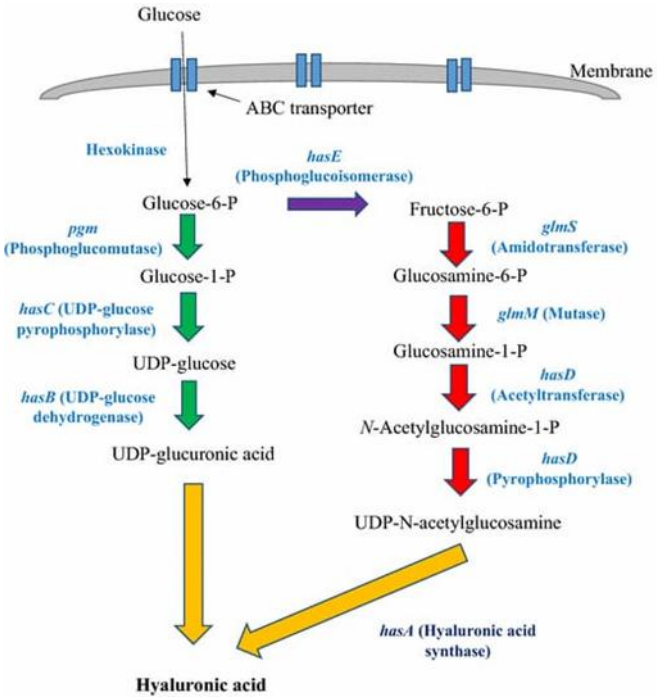

Figure 3: Biosynthesis of HA.

Biosynthesis: The cellular synthesis of HA is a unique and highly controlled process. Most glycosaminoglycans are made in the cell's Golgi networks. HA is naturally synthesized by a class of integral membrane proteins called hyaluronan synthases, of which vertebrates have three types: HAS1, HAS2, and HAS3. Secondary structure predictions and homology modeling indicate an integral membrane protein (IMP). An integral membrane

protein is a protein molecule (or assembly of proteins) that in most cases spans the biological membrane with which it is associated (especially the plasma membrane) or which, is sufficiently embedded in the membrane to remain with it during the initial steps of biochemical purification (in contrast to peripheral membrane proteins). Hyaluronan synthase enzymes synthesize large, linear polymers of the repeating disaccharide structure of hyaluronan by alternate addition of glucuronic acid and N-acetylglucosamine to the growing chain using their activated nucleotide sugars (UDP = glucuronic acid and UDP-N-acetylglucosamine) as substrates.[6]

Mechanism of action: Although the predominant mechanism of HA is unknown, *in-vivo*, *in-vitro*, and clinical studies demonstrate various physiological effects of exogenous HA. Hyaluronic acid possesses a number of protective physiochemical functions that may provide some additional chondroprotective effects *in-vivo* and may explain its longer-term effects on articular cartilage. Hyaluronic acid can reduce nerve impulses and nerve sensitivity associated with pain. In experimental osteoarthritis, this glycosaminoglycan has protective effects on cartilage; exogenous hyaluronic acid is known to be incorporated into cartilage.[7] Exogenous HA enhances chondrocyte HA and proteoglycan synthesis, reduces the production and activity of proinflammatory mediators and matrix metalloproteinases, and alters the behavior of immune cells. These functions are manifested in the scavenging of reactive oxygen-derived free radicals, the inhibition of immune complex adherence to polymorphonuclear cells, the inhibition of leukocyte and macrophage migration and aggregation and the regulation of fibroblast proliferation. Many of the physiological effects of exogenous HA may be functions of its molecular weight. Hyaluronan is highly hygroscopic and this property is believed to be important for modulating tissue hydration and osmotic balance.[8] In addition to its function as a passive structural molecule, hyaluronan also acts as a signaling molecule by interacting with cell surface receptors and regulating cell proliferation, migration, and differentiation. Hyaluronan is essential for embryogenesis and is likely also important in tumorigenesis. Hyaluronan functions are diverse. Because of its hygroscopic properties, hyaluronan significantly influences hydration and the physical properties of the extracellular matrix. Hyaluronan is also capable of interacting with a number of receptors resulting in the activation of signaling cascades that influence cell migration, proliferation, and gene expression.[9]

Pharmacokinetics: The normal systemic kinetics of HA is well established in several species including man. The removal of HA from the circulation is very efficient, with a half-life of 2–6 min and a total normal turnover of 10–100 mg/day in the adult human. The main uptake from the blood takes place in the liver endothelial cells. However, evidence for a role of the kidney in the elimination of HA is accumulating.[10] Recently published data suggest that the elimination kinetics of HA from the systemic circulation may be influenced by a number of factors, such as saturation of the elimination caused by an increased lymphatic input of HA to the circulation, alteration of the blood flow over the eliminating organ and competition with other macromolecular substances such as chondroitin sulphate or proteoglycans. Many of these factors may be operative during different disease states, and may therefore partly explain the observed differences between normal and pathological HA kinetics. The normal and pathological turnover of hyaluronan from the circulation has been determined in many different species, including man by many different authors using different techniques.[11]

Absorption rate and concentration in plasma: After i.v. injection of a bolus dose of [^{14}C]-HA in rabbits, it was shown that 98% of the administered dose had disappeared from the systemic circulation within 6 h after the administration. Similar results were also obtained in man, where 55% and 85% of the acetyl content after i.v. injection of [^{3}H] HA, was completely oxidized after 3 h and 24 h, respectively. It is known that the major part of the elimination of HA from the blood circulation takes place in the liver via receptor-mediated endocytosis in the sinusoidal liver endothelial cells.[12]

Distribution: HA is widely distributed in body tissues and intracellular fluids, including the aqueous and vitreous humour, and synovial fluid; it is a component of the ground substance or tissue cement surrounding cells. It is not known whether hyaluronate sodium is distributed into breast milk.[13]

Excretion (elimination): Renal excretion. By direct measurement of HA in urine it can be calculated that approximately 1% of the normal daily turnover of HA from the systemic circulation in man is filtered via the kidneys. Similar results were obtained in studies on man and in a study on rabbits. Recently, the extraction ratio and clearance over the kidney in pig were reported to be 14% and 41 ml per min, using the method of measuring directly over the organ. In this study, it was also determined that the renal clearance was approximately three times the urinary clearance.[14]

Hepatic elimination: Direct measurement of the difference of the endogenous concentration over a specific organ and knowledge of the blood flow enables calculation of the extraction ratio or clearance directly over a specific organ. By use of this method determined the hepatosplanchnic extraction ratio and clearance of hyaluronan in man to be 33% and 250 ml/min, respectively. The hepatic extraction ratio and clearance have also been determined in pigs by measurement directly over the organ and were found to be 23% and 150 ml/min, respectively. In a similar study on pigs, using the same method of direct determination, the extraction ratio and clearance over the liver were

determined to be 49% and 332 ml/min, respectively. The reason for the discrepancies between these two studies is not known GIT excretion. The total amount of excretion into bile within 24 h was reported to be very low, 0.7% of the administered dose. Similarly, the total amount of excretion into feces, within 100 h of administration, was also very small, about 0.5% of the administered dose.[15]

Overview Information: Hyaluronic acid is a substance that is naturally present in the human body. It is found in the highest concentrations in fluids in the eyes and joints. The hyaluronic acid that is used as medicine is extracted from rooster combs or made by bacteria in the laboratory. The FDA has approved the use of hyaluronic acid during certain eye surgeries including cataract removal, corneal transplantation, and repair of a detached retina and other eye injuries. It is injected into the eye during the procedure to help replace natural fluids. The FDA has also approved the use of hyaluronic acid for injection into the knee for patients with knee osteoarthritis. People use hyaluronic acid for various joint disorders, urinary tract infections (UTIs), acid reflux, dry eyes, vaginal pain, aging, and many other conditions, but there is no good scientific evidence to support these uses.[16]

How does it work? Hyaluronic acid works by acting as a cushion and lubricant in the joints and other tissues. In addition, it might affect the way the body responds to injury.

Likely Effective for

Cataracts. Injecting hyaluronic acid into the eye is effective when used during cataract surgery by an eye surgeon. Swelling (inflammation) and sores inside the mouth (oral mucositis). Hyaluronic acid is effective for treating mouth sores when applied as a gel or used as a rinse.

Possibly Effective for

Aging skin. Some research shows that injecting a specific hyaluronic acid product (Juvéderm Ultra Plus, Allergan) into facial wrinkles can reduce wrinkles for up to one year. Also taking a product containing hyaluronic acid and other ingredients (GliSODin Skin Nutrients Advanced Anti-Aging Formula) by mouth seems to decrease wrinkles and damage from the sun when used for 3 months.

Dry eye. Most research shows that using eye drops containing hyaluronic acid up to 8 times a day helps to relieve symptoms of dry eye.

Osteoarthritis. Hyaluronic acid can be injected into the joint by a healthcare provider to reduce joint pain and stiffness. Hyaluronic acid is approved by the FDA for this condition. But not all people seem to benefit from this treatment. Also, any improvement is usually short-term. Having hyaluronic acid injected into the joint is not recommended for most people with osteoarthritis, but can be discussed with your doctor. Some early research shows that taking hyaluronic acid by mouth might reduce pain in some people with osteoarthritis. But not all research agrees.[17]

Insufficient Evidence for

- Foot sores in people with diabetes. Research shows that applying products containing hyaluronic acid and other ingredients helps heal diabetic foot ulcer compared to regular treatment. It's not known if this benefit is due to hyaluronic acid or other ingredients.
- Eye trauma. Some research suggests that hyaluronic acid might be injected into the eye to treat detached retina or other eye injuries.
- Shoulder pain in people after stroke (hemiplegic shoulder pain). Early research shows that injecting hyaluronic acid might improve pain in people with hemiplegic shoulder pain.
- Nasal surgery. Early research in people who have had nasal surgery shows that using a hyaluronic acid nose wash might improve sinus scarring and crusting better than a salt-water nose wash.
- A disorder that affects the bones and joints, usually in people with selenium deficiency (Kashin-Beck disease). Early research shows that injecting hyaluronic acid might improve pain in people with Kashin-Beck disease.[18]
- Ear infection (otitis media). Early research shows that using a nose wash product containing hyaluronic acid might help to prevent ear infections in children with chronic ear infections.
- Swelling (inflammation) of the nasal cavity and sinuses (rhinosinusitis). Early research suggests that using a nose wash containing hyaluronic acid helps to treat symptoms of sinus infections in people who are also taking antibiotics and steroids.
- Infections of the kidney, bladder, or urethra (urinary tract infections or UTIs). Research shows that injecting hyaluronic acid with chondroitin sulfate directly into the bladder can reduce the number of UTIs in women with frequent UTIs. Some research also suggests that taking hyaluronic acid by mouth with other ingredients, while also using a vaginal gel containing estrogen, helps to prevent UTIs in women with recurring UTIs.
- Thinning of vaginal tissue (vaginal atrophy). After menopause, vaginal tissue gets thinner. Early research suggests that applying a solution containing hyaluronic acid to the vagina helps to reduce burning, itching, and painful intercourse in women with thinning vaginal tissue.
- Wound healing. Early research shows that applying hyaluronic acid to the skin might be helpful for treating burns and skin wounds.

Muscle soreness caused by exercise. Joint pain. Persistent heartburn. Wrinkled skin. Other conditions.

Side Effects & Safety

When taken by mouth: Hyaluronic acid is LIKELY SAFE when used appropriately. Rarely, it may cause allergic reactions.

When applied to the skin: Hyaluronic acid is LIKELY SAFE when used appropriately. Rarely, it may cause allergic reactions.

When given as a shot: Hyaluronic acid is LIKELY SAFE when used appropriately. Hyaluronic acid can cause redness and soreness when injected into the joint. When applied into the eye: Hyaluronic acid is LIKELY SAFE when used appropriately. Hyaluronic acid can increase eye pressure when injected into the eye. But this usually resolves within 48 to 72 hours.[19]

Special Precautions & Warnings

- **Pregnancy:** Hyaluronic acid is POSSIBLY SAFE when given by injection when pregnant. However, there isn't enough reliable information to know if hyaluronic acid is safe to take by mouth or apply to the skin when pregnant. Stay on the safe side and avoid use.
- **Breast-feeding:** Hyaluronic acid is POSSIBLY SAFE when given by injection when breast feeding. But researchers do not know if it affects breast milk and what effect that might have on an infant. There isn't enough reliable information to know if hyaluronic acid is safe to take by mouth or apply to the skin when breastfeeding. Stay on the safe side and avoid use.
- **Hardening of skin and connective tissue (scleroderma):** Applying hyaluronic acid to the skin might make skin ulcers worse in people who have a condition called scleroderma. If you have scleroderma, don't use hyaluronic acid on your skin.

Dosing

The following doses have been studied in scientific research

ADULTS

BY MOUTH: **For aging skin**: A specific product (GliSODin Skin Nutrients Advanced Anti-Aging Formula, Isocell North America Inc.) containing krill oil, sea buckthorn berry oil, cacao bean extract, and hyaluronic acid, has been used daily for 90 days.

For osteoarthritis: A specific product (Oralvisc, Bioibérica) 80 mg has been used daily for 3 months.

APPLIED TO THE SKIN:

For dry eye: Eye drops (Hyalistil, Hyalein, New Hyaluni, Hyaluni, Visaid) containing 0.1% to 0.3% hyaluronic acid have been used 3-8 times daily.

For swelling (inflammation) and sores inside the mouth (oral mucositis): Hyaluronic acid (Gelclair, Helsinn Healthcare SA) can be mixed with water and used as a mouth rinse.

BY INJECTION

- **For aging skin:** Healthcare providers can inject a hyaluronic acid product (Juvéderm Ultra Plus, Allergan) into skin wrinkles.
- **For osteoarthritis:** Healthcare providers can inject hyaluronic acid into the joint.

CONCLUSION

Hyaluronic acid has been used for more than 20 years in many products throughout the world. Because of its biocompatibility, biodegradability, and readily modified chemical structure, HA has been extensively investigated in drug-delivery applications. A variety of commercially available preparations of HA derivatives and cross-linked HA materials have been developed for drug delivery; these materials are created in forms such as films, microspheres, liposomes, fibers, and hydrogels. Through multidisciplinary discoveries about the structure, properties, biological activity, and chemical modification of this unique polymer, HA has found success in an extraordinarily broad range of biomedical applications. Future clinical therapies of HA-derived materials critically rely on a more detailed understanding of the effects of HA molecular weight and concentration and how this biomolecule specifically interacts with cells and ECM components in the body. The increased use of these materials will require finely tuned and controllable interactions between HA and its environment. Work in these areas is underway; for example, adhesive peptide sequences have been covalently bound to HA materials. Also, environmentally responsive materials have been synthesized from HA. These materials can be created to swell or degrade in response to inflammation, electrical stimulation, and heat.

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