Chemo-physical Properties and Biomedical Applications of Hyaluronic Acid in Medicine

DANUT VASILE[1], RALUCA IANCU[2,*], CAMELIA BOGDANICI[3*], EMIL UNGUREANU[4], DANA CIOBOTEA[5], RADU CIULUVICA[4], GEORGE IANCU[6]

[1] Carol Davila University of Medicine and Pharmacy Bucharest, Faculty of General Medicine, Surgery Department, 37 Dionisie Lupu Str., 020021, Bucharest, Romania

[2] Carol Davila University of Medicine and Pharmacy Bucharest, Faculty of General Medicine, Ophthalmology Department, 37 Dionisie Lupu Str., 020021, Bucharest, Romania

[3] Grigore T. Popa University of Medicine and Pharmacy Iasi, Faculty of General Medicine, Ophthalmology Discipline,16 Universitatii Str., 700115, Iasi, Romania

[4] Carol Davila University of Medicine and Pharmacy Bucharest, Faculty of General Medicine, Anatomy Department, 37 Dionisie Lupu Str., 020021, Bucharest, Romania

[5] Victor Babes University of Medicine and Pharmacy, Surgery Department, Ophthalmology Discipline, 2 Eftimie Murgu Sq.,, 300041, Timisoara, Romania

[6] Carol Davila University of Medicine and Pharmacy Bucharest, Faculty of General Medicine, Obstetrics and Gynecology Department, 37 Dionisie Lupu Str., 020021, Bucharest, Romania

Hyaluronic acid is a mucopolysaccharide encountered in most body fluids and extracellular matrix. The aim of our review is to summarize current evidence about chemico-physical properties of hyaluronic acid, highlighting biomedical applications of hyaluronan derivatives. It is a glycosaminoglycan made of repeating disaccharide units containing a carboxylate group, four hydroxyl groups and one carboxylate group, with hydrophilic properties. Its particular structure with multiple coils forming an entangled network results in unique pseudoplastic and viscoelastic characteristics. Its viscous and elastic behavior, depending on the applied strain, makes hyaluronan widely applicable in biomedical field. The large amount of functions and applications is determined by the physico-chemical properties, which allows a polymorphism of the hyaluronic acid structures depending on the molecular weight variations, concentration and ionic status. It is currently used in ophthalmology, orthopedics and rheumatology, in plastic surgery, surgery and otolaryngology as well. Already widely used in clinical practice, hyaluronic acid proves to be often the best solution for difficult medical problems. Future developments in nanomedicine and drug delivery linked to hyaluronic acid are emerging.

Keywords: Hyaluronic acid, Glycosaminoglycan, Viscoelastic properties

Hyaluronic acid, also called hyaluronan, was first described in 1934 by Karl Meyer and John Palmer in the *Journal of Biological Chemistry.* They discovered this high weight biopolysacharide in the vitreous of bovine eyes [1].

Hyaluronic acid, representing the main component of the glycosaminoglycans, is a carbohydrate, respectively a mucopolysaccharide, and among the natural polymers it is the most abundant in the human body. It is present in almost all body fluids (eye vitreous humor, synovial fluid), extracellular matrix and pericellular matrix, connective and epithelial tissues [2,3]. Due to its rheological properties hyaluronic acid provides stabilization of the extracellular matrix, water homeostasis, exhibit wound healing properties, making it very appealing for medical use [4].

Chemical properties

Hyaluronic acid is chemically classified as an glycosamino glycan, an extracellular matrix component, alongside with dermatan sulphate, keratan sulphate and heparin sulphate. It is a polyanionic polysaccharide composed of repeating disaccharide units of D-N-acetylglucosamine (GlcNAc) and D-glucuronic acid (GlcUA), which are linked through b(1,4) and b(1,3) glycosidic bonds (fig. 1). Every disaccharide unit comprises one acetamido group, four hydroxyl groups and one carboxylate group, but, unlike the other three glycosamino glycans it does not comprise sulphate group and it is not covalently bound to a proteoglycan core protein [5].

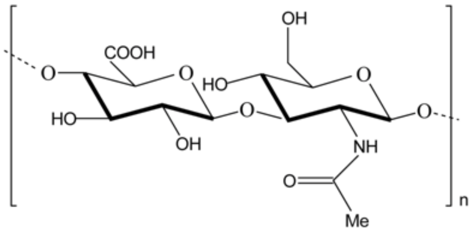

Fig. 1. Chemical structure of Hyaluronic acid

Due to its hydrophilic characteristic, its molecular weight can vary widely (between 10^4 and 10^7 Da) and its function can change depending on its size [6].

Hyaluronic acid is well represented in humans and other vertebrates. It has a high turnover rate, approximately 30% being renewed every day [7]. Despite the fact that the other glycosamino glycans are made in the Golgi apparatus, hyaluronan naturally synthesis takes place in plasma membrane by the HyA synthases (HAS), of which vertebrats have three types: HyAS-1, HyAS-2, HyAS-3 [8,9]. Hyaluronan synthase enzymes produce hyaluronic acid chains by alternate addition of the two substrats GlcNAc and GlcUA, using their activated nucleotid sugars (GlcUA-UDP and GlcNAc-UDP) [10]. Hereby we summarize the chemical formula which leads to hyaluronan synthesis; the number of nucleotide n is usually around 10,000, but it can reach and sometimes excide 20,000.

$$\text{nUDP-GlcUA} + \text{nUDP-GlcNAc} \rightarrow \text{2nUDP} + [\text{GlcUA} + \text{GlcNAc}]_n.$$

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* email: ralucavasile2002@yahoo.com; camelia.bogdanici@gmail.com

Hyaluronan can be extracted from various animal tissues (bovine vitreous humour, rooster combs, shark skin) and can also be produced by microbial fermentation from group C Streptococci (Streptococcis equi, Streptococcus sp, Streptococcus zooepidemicus [11,12]) Escherichia coli,Bacilus sp etc [13].

Physico-chemical properties

Hyaluronic acid exists in an extended, random coil configuration in solution. This helicoidal structure is stabilized by hydrogen bounds which are placed parallel to the axis of the chain (fig. 2).

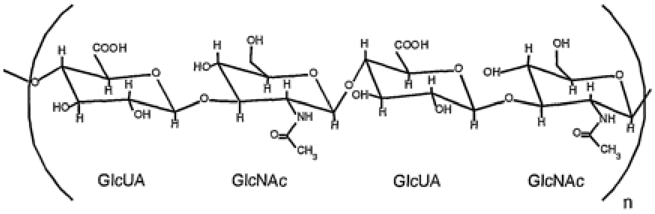

Fig. 2. Hyaluronic acid hydrogen bonding.

At very low concentrations, hyaluronic acid chains form an entangled network in which coils are overlapping, but in dilute state, chains do not interact with each other. This characteristic represents the basis of rheological properties of hyaluronan [14]. Therefore, hyaluronan has pseudoplastic and viscoelastic characteristics, with non-Newtonian flow properties [15].

The characteristics that influence physico-chemical properties of hyaluronan are the molecular weight, concentration, temperature, *p*H, ionic composition of the solvent and binding or non-binding of proteins [16]. Strong influences on rheological properties are determined mainly by molecular weight and concentration, these being approached by multiple authors [15,17,18]. The concentration was found to have a weaker effect on the elastic properties of hyaluronan [17,18]. The viscoelastic properties of the solution containing hyaluronan are influenced by *p*H values; extreme *p*H values (2.5 – 3.5 or above 12.5) reduce significantly the viscosity, as a consequence of decreasing hydrogen bounds. Neutral *p*H is associated with adequate viscosity [19]. The viscoelastic properties are highly influenced by the frequency of applied strain. An elastic behavior of the hyaluronan solution is observed when high frequency stress is applied, while low frequency strain is followed by mainly viscous behaviour [20].

Biomedical applications

The unique properties of hyaluronan and derivated compounds make them widely applicable in biomedical field. Hyaluronic acid is a biopolymer with water-binding ability, being recognized as one of the most hydrophilic molecules in nature. Because of its many carbohydrate subunits, hyaluronic acid is able to absorb large quantities of water and has a gelly-like consistency. This unique bio-characteristic gives it the capacity to lubricate movable parts of the body (muscles, joints) [21].

Hyaluronic acid synthetized in living organisms is identical with the compound obtained by bacterial fermentation. This is why hyaluronan shows unique properties like biocompatibility and non-immunogenicity [22], which makes it suitable for a wide variety of clinical applications. On the other hand HA is highly biodegradable [23], being rapidly lysate by hyaluronidases. Longer in situ stability was obtained by different chemical processes like cross-linking or conjugation [24,25]. By cross-linking HA gets enhanced rheological properties without altering its biocompatible characteristic [26].

Hyaluronic acid network embeds cells, but beside this support role it was demonstrated that hyaluronan interacts with cell differentiation, proliferation, adhesion, motility and tissue repair [27,28]. So all three phases of wound healing (inflammatory phase, proliferative phase, remodeling phase) are influenced by biological properties of hyaluronan [27].

In 2004, Balazs organized the biomedical properties of hyaluronic acid in five main domains [29]. Viscosurgery uses the capacity of hyaluronan to maintain spaces during surgical procedures and to spare fragile tissues like in ophthalmic surgery [30]. Viscoaugmentation is based on the bulking properties of hyaluronan, that enables it to complete spaces and create volume, obstructing leakeage due to incontinent sphincters, correcting glottal malfunction or skin wrinkles [31]. Viscoseparation is based on hyaluronan interfering in wound healing. Used post-surgery it helps avoiding pathological tissue scarring, limiting adhesion formation [30]. Viscosupplementation consists in using hyaluronan as a substitute for tissues with similar rheological proeprties, for example in degenerative arthritis [26,32]. Viscoprotection allows tissues to be protected by hyaluronan against injurious environmental conditions [30].

We list further the major medical fields in which hyaluronic acid is useful and effective:

- *Ophthalmology.* Hyaluronic acid is a major component of the vitreous humor of the eye. Due to its physico-chemical properties, hyaloronan solutions are widely used in ophthalmic surgery for augmenting anatomical spaces (in keratoplasty [33], cataract surgery, vitreoretinal surgery), for protecting ocular tissues (like corneal endothelium during cataract extraction [34]), for replacing vitreous fluid loss [35]. Because it is perfectly biocompatible and non-immunogenic exogenous HA does not negatively influence postsurgical outcomes (like intraocular pressure). Also, HA solutions provide an increased viscosity to eye-drops, being recognized lately as a major component of artificial tears, coating and protecting the ocular surface [36].

- *Orthopedic and rheumatologic application.* Hyaluronic acid preserves the viscoelasticity of synovial fluid and plays an important role in cartilage and tendon augmentation [37, 38, 39]. Due to its high molecular mass and water-binding ability, HA acts at articular level as a lubricant and stress releaser [40]. Injection of high molecular weight HA into the articular space (viscosupplementation) represents an important key in therapeutical management of osteoartfritis since 1990s [41].

- *Surgery and wound healing.* HA influence all phases of wound healing. It is involved in inflammatory response, oedema appearance, angiogenesis debut, granulation tissue formation, epithelialization and remodeling [27,42]. Hyaluronic acid promotes healing of fresh wounds (like postoperative incisions, burns), but also augment chronic wounds (metabolic ulcers) [43].

- *Dermatology, cosmetics and plastic surgery.* Approximately 50% of the HA found in the human body exists in skin. With age, HA production decreases and it concentrates preferentially in dermis than in epidermis. These metabolic alterations promote skin ageing. Delivered topically (HA gels) or by injection route (cross-linked HA), the HA content of the skin can be restored [44, 45].

-Otolaryngology. HA represents an important component of the vocal folds. Due to its biomechanical properties and aforementioned characteristics it influences vocal cords function. HA derivatives are used for viscoaugmentation of the traumatized vocal cords [46].

-Cardiovascular system. HA has antiadherent properties. Therefore, surface-modified cardiovascular implants coated with cross-linked HA have been recognized to prevent postoperative thrombosis by decreasing platelet adhesion [47].

-Hyaluronic acid in cancer cell biology and treatment. Besides the important role of hyaluronic acid in wound healing, in late years it was emphasized its implications in tumoral processes. Carcinomas, melanomas, lymphomas were found to present fragments of hyaluronic acid. On the other hand, specific and unique biological properties enable HA to be a perfect drug carrier and deliverer in anticancer medical management [48,49].

Conclusions

Over the past 50 years international studies gathered information about the chemical properties, rheological characteristics and biomedical applications of hyaluronic acid. It was demonstrated that hyaluronan is actively involved into a multitude of biological processes. The large amount of attributes, functions and applications is determined by the physico-chemical properties which allows a polymorphism of the HA structures depending on the molecular weight variations, concentration and ionic status.

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