

Hyaluronic Acid in the Treatment and Prevention of Skin Diseases: Molecular Biological, Pharmaceutical and Clinical Aspects

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Key Words

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Abstract

The glycosaminoglycan hyaluronic acid (HA), or hyaluronan, is a major component of the extracellular matrix of skin, joints, eye and many other tissues and organs. In spite of its simple structure, HA demonstrates remarkable rheological, viscoelastic and hygroscopic properties which are relevant for dermal tissue function. Biological activities in skin, however, are also due to its interaction with various binding proteins (hyaladherins). Due to an influence on signaling pathways, HA is involved in the wound-healing process and scarless fetal healing. Increased HA concentrations have been associated with inflammatory skin diseases. In clinical trials, topical application of HA improved wound healing; in particular, acute radioepithelitis, venous leg ulcers or diabetic foot lesions responded to HA treatment. Moreover, as a topical drug delivery system for diclofenac, an HA gel has recently been approved for the treatment of actinic keratoses. Finally, chemical modifications led to new HA derivatives and biomaterials, which may be introduced into

therapy in the future. Therefore, ongoing research offers new horizons for the therapeutic use of this glycosaminoglycan which has been regarded as an inert structural component until recently.

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Introduction

The glycosaminoglycan hyaluronic acid (HA), also known as hyaluronan, can be found in many tissues and body fluids of mammals, with the highest concentrations in connective tissue and skin, which also harbor most of the body's amount. Although detected already 50 years ago in the extracellular matrix, synovial fluid of joints and in the vitreous body of the eye and first regarded as an inert filling material, HA functions are still the subject of ongoing research. Indeed, new biological and pharmaceutical aspects became apparent during the last two decades, and nowadays HA is widely utilized in ophthalmology, rheumatology and dermatology because of specific effects, too. Clinical use in dermatology now encompasses HA as a biomaterial for bioengineering purposes or temporary dermal filler in aesthetic dermatology, but also for the stimulation of wound healing as well as drug vehicle in

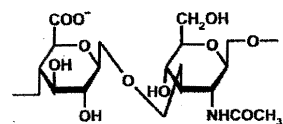
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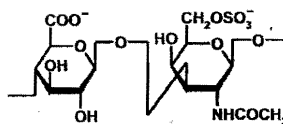
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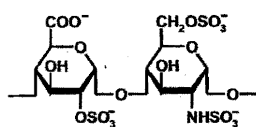
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Hyaluronan



Chondroitin-6-sulfate



Heparin

Fig. 1. Disaccharide structure of hyaluronan, chondroitin-6-sulfate and heparin. Hyaluronan is composed of repeating disaccharide units containing *D*-glucuronate and *N*-acetylglucosamine. Chondroitin-6-sulfate consists of *D*-glucuronate and *N*-acetylglucosamine-6-sulfate and heparin of *D*-glucuronate-2-sulfate and *N*-sulfo-*D*-glucosamine-6-sulfate. Unlike other glycosaminoglycans which possess α - and β -linkages, hyaluronan and chondroitin-6-sulfate have exclusively β -linkages, thus endowing them with more resistance to degradation.

topical formulations [1–6]. This article reviews the broad spectrum of biological, pharmaceutical and clinical aspects of HA.

Molecular Biology of Hyaluronan

Chemistry, Metabolism and Biosynthesis

HA belongs to the family of glycosaminoglycans, which also includes dermatan sulfate, chondroitin sulfate, keratan sulfate, heparan sulfate and heparin. HA is an unbranched polysaccharide built up from repetitive units of *D*-glucuronic acid and *D*-*N*-acetylglucosamine disaccharides (fig. 1). In contrast to other glycosaminoglycans,

there are no sulfoesters. An average HA chain contains up to 10,000 disaccharide repeats with a molecular weight ranging from 2×10^5 to 10^7 Da. Due to hydrogen bonding of adjacent sugar units and mutual repulsion between carboxyl groups, HA forms a stiffened and expanded random coil in solution.

Concentration and mean molecular weight of HA determine its rheological properties. High viscosity at low shear rates and high elasticity at high shear rates are typical of pseudoplastic molecules. These attributes are relevant for the structural function of HA in connective tissue and the cushioning and lubricating effects in the aqueous humor of the eye and synovial fluid [7].

Facing the function as relatively inert structural element of connective tissue and cartilage, the turnover of HA is extraordinarily rapid. The half-life is 2–5 min in the blood, 12 h in skin and 1–3 weeks in cartilage. Cleavage is due to hyaluronidases, chondroitinases and hexosaminidases [7] or by exposure to oxygen free radicals [8, 9]. The rapid turnover suggested that HA may induce specific effects, too.

Initially, HA was isolated from bovine vitreous humor and human umbilical cord, and shortly thereafter from streptococci and rooster combs in a highly purified and high-molecular-weight form. Other than most glycosaminoglycans which are synthesized within the Golgi network and bound to protein cores, the free linear HA polymer is built up at the inner side of the cell membrane and directly secreted into the extracellular space [10]. In vertebrates, 3 HA synthases have been characterized, which synthesize HA chains of different average length and function [11].

Water Binding

As mentioned above, HA is most abundant in skin, dermis, viable epidermis and stratum corneum [12]. Due to the high water-binding capacity, HA should contribute to the maintenance of the extracellular space, facilitate the transport of nutrients and ion solutes. Solutions of the hygroscopic macromolecule HA are highly osmotic, e.g. they serve as an osmotic buffer in the kidney. In skin, this property is likely to be relevant in controlling tissue hydration during an inflammatory process but also during embryonic development [1–5]. Since HA also preserves tissue hydration in skin, it is often used as moisturizing agent in cosmetic formulations.

Cell Receptors and Binding Proteins (Hyaladherins)

As described, the high turnover rate indicates additional roles of this glycosaminoglycan, and despite its sim-

ple structure HA displays a wide range of biological activities and can act as a signaling molecule, too. As with ceramides, which were first described as important components of the epidermal barrier [13], the important function in cell signaling has been revealed only recently [14]. Other groups of biological agents underestimated as cell linkers and now known for signal transducing are integrins, cadherins and selectins [15].

The hyaladherins comprise a large family of proteins able to bind HA. Hyaladherins can be grouped either according to their cellular and extracellular location or according to the amino acid sequence by which they bind HA. This binding domain, also termed link module, is structurally similar among many HA-binding proteins of the extracellular matrix (ECM), such as the chondroitin sulfate proteoglycans aggrecan, brevican, versican and neurocan, also called hyalactans. Furthermore tumor necrosis factor-stimulated gene 6 product, another extracellular hyaladherin, and the cell surface HA receptors CD44 and lymphatic vessel endothelial hyaluronan receptor 1 are members of this link module superfamily [16, 17]. One of the best characterized hyaladherins is CD44 [18], a widely distributed cell surface protein existing in several isoforms. CD44 participates in various cellular functions, such as cell aggregation, retention of pericellular matrix, matrix-cell and cell-matrix signaling, receptor-mediated internalization and degradation of HA, angiogenesis, cell proliferation and activation. Another HA-binding protein, RHAMM (receptor for hyaluronan-mediated motility) lacks this link module; RHAMM, too, occurs in multiple forms at the cell surface but also intracellularly [18]. This protein mediates cell locomotion in a wide variety of mobile cells and is associated with migration and metastasis. Furthermore, HA binds to intercellular adhesion molecule 1 (ICAM-1), also designated as CD54, which was originally described as a metabolic receptor for HA. However, ICAM-1 is also widely distributed on endothelial cells and leukocytes, and binding of HA may contribute to the control of ICAM-1-mediated inflammatory activation [19]. The growing family of hyaladherins, their structural organization and biological activities are described in more detail elsewhere [20].

Cell Signaling

Over the last few years numerous studies on the biological effects of low- and high-molecular-weight HA (HMWHA) have been published. While small-molecular-weight HA or HA fragments have been implicated in various biological processes including cell proliferation, angiogenesis, migration, maturation, activation of protein

tyrosine cascades and proinflammatory activity [21–25], HMWHA seems to be involved to a much lesser extent or displays contrary effects, e.g. HMWHA chains inhibit cell proliferation, whereas shorter polymers stimulate cell proliferation [21]. Another study demonstrated that binding of HMWHA to CD44 results in receptor clustering, and binding was inhibited by addition of HA-derived oligosaccharides [22]. Very recently, it was shown that HA fragments, which are released following injury, increase the expression of the chemokine IL-8 in endothelial cells, hence stimulating the endothelium to recognize injury and initiate wound repair [25]. The release of IL-8 was dependent on Toll-like receptor 4, a member of a novel receptor family which plays a critical role in innate immune recognition of pathogens [26] and now appears to be involved also in recognition of tissue damage.

Radical Scavenger

In the skin HA might also act as a scavenger of free radicals and antioxidant under physiological conditions. HMWHA forms a viscous, pericellular meshwork around cells in a dose-dependent manner, restricting movement of reactive oxygen species [27]. Moreover, recent spectroscopy studies allowing enzymatic digestion of HA indicated the presence of a double bond in the *D*-glucuronic acid unit [28] which can form a complex with reactive oxygen species and reduce the toxicity of radicals. Exposing liposomal skin lipids to UV irradiation, HA and its fragments exerted antioxidative effects [29] which supports the influence of the function as a radical scavenger.

Wound Healing and Skin Diseases

During wound healing, specific regulation of HA synthesis and catabolism is responsible for various cellular responses by interacting with HA receptors [30]. Briefly, in the early inflammatory phase HA accumulates in the wound tissue, and interacting with CD44 induces proinflammatory cytokines and enhances cell infiltration. The antioxidant properties of HA appear to moderate the inflammation and prevent oxygen free radical damage of granulation tissue [31]. During the granulation phase, elevated HA levels promote cell proliferation, cell migration, e.g. of keratinocytes, and angiogenesis.

Besides this, a prolonged presence of HA may also contribute to scarless fetal tissue repair. The HA content in fetal wounds remaining high for longer periods than in adult wounds and the corresponding hyaluronidase levels being relatively low lead to the suggestion that HA may, at least in part, reduce collagen deposition and scarring [32].

Increased concentrations of HA in serum are associated with several inflammatory skin diseases, such as psoriasis [33, 34], progressive systemic sclerosis [35–37] and dermatomyositis [38]. Furthermore, elevated concentrations of HA in suction blister fluid [39] were found in blisters raised on psoriatic lesions. Histochemical staining showed high expression of ICAM-1, especially close to the vessels in psoriatic skin [19].

Pharmaceutical Aspects

Drug Delivery

As a biodegradable, nonimmunogenic and nontoxic compound, even at very high concentrations, HA has found important applications in drug delivery. On topical application the physicochemical properties of HA appear to increase the residence time of other agents. An *in vitro* Franz cell study showed that after 7 days more diclofenac was retained in the epidermis after application in the hyaluronan formulation (41%), as compared to 25% with the buffer control [40]. Moreover the efficacy of nonsteroidal anti-inflammatory drugs and cyclosporine A applied topically to the skin is considerably enhanced, although the exact mode of action is still unclear. Since the HA receptors CD44, RHAMM and ICAM-1 are upregulated at inflammatory sites and neoplasia, specific binding of HA may enhance the delivery of these and probably other agents to the site of disease [19, 41–43]. Recently, a 3% diclofenac/2% HA gel has been approved for the treatment of actinic keratoses (AKs) due to the outcome of various *in vitro* [40, 44–45] and animal studies [46, 47] as well as clinical trials [48, 49].

Epidermal-growth-factor-loaded liposomes anchored with HA on the surface suggested HA to act as site-adherent and sustained-release carrier of drugs for the topical therapy of wounds and burns [50]. In this early study, epidermal growth factor served as a test drug and a human epidermoid carcinoma cell line as model of an *in vivo* target. The peptide was released continuously with similar first-order kinetics from surface-modified and nonmodified liposomes; however, only the HA-modified liposomes demonstrated bioadhesive properties binding with high affinity to the cell line.

Chemical Modifications

Derivatization of HA and HA-drug conjugates offers a number of advantages over simple drug-HA mixtures. Chemical modification allows to tailor the physicochemical properties of the glycosaminoglycan according to the

desired applications and can have a significant impact on the turnover and clearance of the HA derivate. HA conjugates may improve water solubility relative to the parent drug, and HA-drug hydrogels appear to localize a slow-release formulation at a specific site in the body [51].

To produce novel biomaterials with desirable physicochemical, mechanical and biocompatible properties, HA has been blended with other materials. This includes blends with polyvinyl alcohol for ophthalmic use, with carboxymethylcellulose (cross-linked with carbodiimide) to produce a bioabsorbable film for the prevention of postsurgical adhesions, and for wound-healing applications with collagen [52].

Clinical Use of Hyaluronan

The remarkable properties and biocompatibility of HA have led to its use in a number of clinical applications. Exogenous application of HA was shown to provide a beneficial effect in wound healing and tissue engineering and in topical drug delivery.

Application as Topical Agent

Besides the use in many cosmetic products, several clinical studies have been performed with HA applied as cream or dressing. In radiotherapy, severe skin reactions like acute radioepithelitis, observed often in the beginning of the treatment, not only reduce the patients' quality of life but also their compliance. A randomized, double-blind, placebo-controlled study using HA cream 0.2% (Ialugen®) was carried out in 134 patients to investigate the prophylactic use of HA after radiotherapy treatment [53]. Twice daily application of HA cream causes only a slight delay of the onset of acute skin reactions but significantly reduces their intensity compared with placebo cream. Additionally, the healing process using HA cream appears accelerated. In a multicenter controlled study 50 patients with venous leg ulcers received 1 HA gauze pad a day in comparison with dextranomer paste for 3 weeks. Local tolerability of both treatments was excellent, and although in both groups appearance and dimensions of ulcers significantly improved, there was a faster and greater reduction following HA [54]. A pilot study evaluating the use of a HA-containing dressing (Hyalofill®) in the treatment of diabetic foot ulcers has recently been conducted [55]. All 36 patients first received surgical debridement for their diabetic foot wounds and then HA dressing until the wound bed approached 100% granulation tissue, followed with a moisture-retentive dressing until wound closure.

The authors concluded that HA-containing dressings might be useful in diabetic foot ulcer care. Another uncontrolled, pilot trial assessed the efficacy of Hyalofill F (a partial benzyl ester derivative of HA) in the therapy of venous leg ulcers. The treatment of 20 patients showed a promising healing process and was well tolerated [56]. Again, additional clinical trials including a control group need to be performed. Moreover, in nonhealing diabetic foot lesions, transplantation of autologous keratinocytes cultured on membranes composed of a benzyl ester of HA (Laserskin® autograft) appears to aid wound healing [57]. This study confirmed previous findings from two clinical case reports and a pilot study, applying HA membranes as delivery system for autologous keratinocytes [58, 59].

Application as Topical Drug Delivery System

AKs are relatively common skin lesions mainly induced by extensive exposure to UV radiation in sunlight leading to mutations in the telomerase gene and p53 tumor suppressor gene. The potential of AK developing into invasive squamous cell carcinoma is generally accepted and underlines the necessity for early treatment of AK lesions. Several treatment options exist for AK including surgical, physical, photodynamic and drug therapies [60]. The approval of topical 3% diclofenac in 2.5% HA gel (Solaraze®), both in the USA and in Europe, offers a new, effective and well-tolerated pharmacological approach. Various studies [48, 49] evaluated the efficacy in patients with AK and have been reviewed recently [61]. In summary, diclofenac HA gel significantly reduces the numbers of AK lesions if applied twice daily for 60 or 90 days and can fully clear lesions. The most common adverse effects like pruritus, dry skin and skin exfoliation were mainly related to the application site and mild to moderate in severity. In the literature 2 cases of allergic contact dermatitis associated with topical diclofenac HA gel have been reported, although patch testing showed that this adverse reaction was solely due to sodium diclo-

fenac and polyethylene glycol monomethyl ether 350, respectively [62, 63].

In a randomized controlled trial topical application of recombinant tissue plasminogen activator in 1% HA appeared to be a promising agent for treating venous ulcers [64]. However, additional trials to confirm these findings are lacking so far.

Outlook and Perspectives

In the present article, we have discussed the biological and physicochemical properties of this remarkable biopolymer and its applications in the treatment of skin diseases. However, much work needs to be done to elucidate the multifaceted role of HA. One challenge and important aspect of HA biology is the use of often poorly controlled HA-based reagents [65] and difficulties in accurate determination of quantity and molecular masses in biological samples. Furthermore HA is obtained from different sources and may contain impurities, and current preparations are not characterized thoroughly [66]. New developments in electrophoretic and chromatographic techniques [67] allowing to select HA of well-defined size may motivate future investigations on biological activities and thus result in additional indications. In terms of current clinical use, one is tempted to speculate whether HA may cause adverse reactions upon application of preparations with different polymer sizes owing to its recently discovered function as signaling molecule. Although HA acts no longer solely as an inert structural material, reports regarding this issue have not been published yet.

From its initial purification from bovine vitreous 70 years ago, onward research on HA was cumbersome, with progress often impeded by failures. Nonetheless the biomedical applications of HA today are numerous and will expand undoubtedly in the near future.

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