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Review article

Hyaluronan as a promising excipient for ocular drug delivery

Michaela Guter, Miriam Breunig *

Department of Pharmaceutical Technology, Faculty of Chemistry and Pharmacy, University of Regensburg, 93049 Regensburg, Germany

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ABSTRACT

Hyaluronan (HA) is a naturally occurring polysaccharide and well known for its exceptional properties such as high biocompatibility and biodegradability, along with a low immunogenicity. Besides its use for various biomedical applications it recently came into focus as a favorable excipient for the formulation of various ocular therapeutics. This review article summarizes the ocular distribution of HA and its most heavily investigated binding protein "cluster of differentiation 44" (CD44) which is the rationale for the clinical use of HA, primarily as an additive in ocular applications ranging from eye drops to contact lenses. Moreover, examples will be given for using HA in various pre-clinical approaches to generate entirely new therapeutics, most notably in the field of nanotechnology.

Contents

1. Introduction

Ocular diseases are numerous and diverse. Some present few symptoms or a mild progression, while others may be associated with pain, double vision, inflammation, and ultimately vision loss. In the past decade, significant progress has been achieved toward a better understanding of the pathogenesis and genetics of many ocular diseases such as glaucoma or ocular neovascularization (e.g., age-related macular degeneration (AMD) or diabetic retinopathy (DR) [1]). Over the same time period, the field has seen a substantial expansion of both therapeutic options and routes of delivery. For many years, ocular medicines have been applied predominantly as eye drops. Despite their potency, these medications

* Corresponding author.
E-mail address: miriam.breunig@chemie.uni-regensburg.de (M. Breunig).

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have a long list of drawbacks, including poor compliance, inadequate application by patients, poor bioavailability, and systemic side effects [2]. In addition, these therapeutic options fail at treating diseases that originate in the posterior segment of the eye. The emergence of biologics has helped fill this therapeutic gap; intravitreal injections are now routine procedures [3]. In addition, research and development on novel drug delivery materials and strategies for therapeutics, ranging from small molecules to biologics and nucleic acids, have advanced the field tremendously (for reviews please refer to [4–8]).

More recently, the polysaccharide HA has come into focus as a favorable excipient for the formulation of various ocular drug delivery systems. HA is a naturally occurring structural component of the extracellular matrix (ECM) and can be gained by extraction from animal tissues such as the rooster comb but also via bacterial fermentation in Streptococci or Bacilli [9,10]. Its non-immunogenic, non-fouling and favorable physicochemical properties (e. g. high water binding capacity, pseudoplasticity, optical transparency) have made the use of HA in biomedical applications quite popular. For example, it has been applied as an artificial lubricant in joints [11], a substitute material for the vitreous body [12], and a scaffold material for tissue engineering [13]. Additionally, HA has contributed to the development of cancer diagnostics and therapy [14,15]; for instance, HA-based hydrogels have been used for local delivery of macromolecular drugs (e.g., for interferon-α2 [16,17], trastuzumab [18], and nucleic acids [19]). HA-drug conjugates have also shown promise when formulated to improve drug solubility and stability, in a similar fashion to PEGylation [20]. Lastly, HA has been used successfully as a targeting sequence to guide drug-loaded nanoparticles (NP) to their final destination [14]. Despite these efforts, HA-based excipients are just beginning to garner recognition as powerful catalysts for new drug delivery applications in the field of ocular pathology.

Here, we first present a general overview of HA and its properties. Then, we describe the distribution of HA and its binding protein CD44 in the anterior and posterior segment of the eye. Finally, we provide a critical review of recent developments in the field of HA-assisted ophthalmic drug delivery and highlight HA's potential therapeutic effects.

2. HA and its interaction with the CD44 receptor

HA is the main component of the ECM and can therefore be found ubiquitously in the human body. The largest amount is present in the skin, which accounts for over 50% of total body HA [14]. However, the concentration is also high in the vitreous body, from which it was originally isolated in 1934 [21]. Its significant role in the body is underlined by its high synthesis rate and an extremely rapid turnover of 5 g per day in an adult of 70 kg [22].

HA is composed of alternating units of D-glucuronic acid and N-acetyl-glucosamine (Fig. 1). The monomers are linked via β-1,4- and β-1,3-glycosidic bonds and form a long, linear polymer chain. The pK_a value of the carboxylic acid groups is between 3 and 4 [23]. Consequently, the polysaccharide is negatively charged at physiological pH. Native HA is present as high molecular weight (MW) material up to 10^7 kDa and is well known for being biocompatible, biodegradable, non-toxic, and non-immunogenic [24]. Although the chemical structure is very simple, HA exhibits exceptional properties and various biological functions [25]. Due to its hydrophilic character and long chain length, HA can bind enormous amounts of water and may thus swell up to a volume 1000-fold greater than its original solid volume [14,26]. HA solutions are viscous and characterized by shear thinning and pseudo-plastic flow behavior [27]. Consequently, the polymer is ideally suited as a lubricant in several biological functions and was rapidly deployed as an artificial viscosupplement, which diminishes friction and abrasion in the joint gap [28].

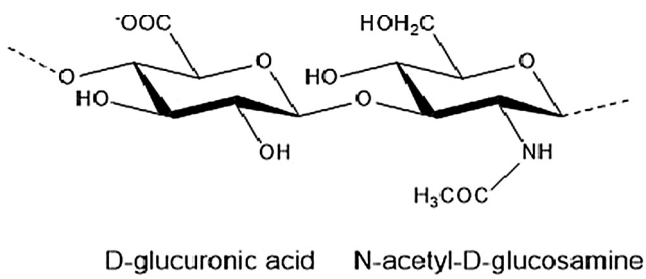

Fig. 1. Chemical structure of HA. HA is a natural heteropolysaccharide composed of the repeating disaccharide unit D-glucuronic acid/N-acetyl-D-glucosamine. The monomers are linked via β-1,4- and β-1,3-glycosidic bonds until the molecular weight reaches values of up to 10^7 kDa.

HA promotes the structure formation and hydration of the ECM throughout various tissues in the body and enables their mechanical functionality and stability [26,29]. Interactions with several extracellular binding proteins (e.g., versican, aggrecan, and neurecan), distributed unevenly across tissues, strengthen the structure of the matrix [30]. In addition, hydration and electrostatic repulsion of anionic carboxyl groups on the polysaccharide chain contribute to the expansion of the polymer [25]. The resulting pressure caused by the swelling is strong enough to separate neighboring tissues, allowing cells to migrate within the newly emptied spaces. This HA-induced "cell migration highway" is of special importance in tissue development, wound healing, and cancer metastasis. Cellular receptors for HA such as CD44, receptor of hyaluronan mediated mobility (RHAMM) and lymphatic vessel endothelial hyaluronan receptor 1 (LYVE-1) link cells to the ECM, mediate cellular mobility, transduce signals from the extracellular environment to the intracellular space and are responsible for HA internalization and degradation. Some HA-binding proteins share a structural domain known as "link module" but other binding sites have also been identified [31].

The most heavily investigated of the HA-binding proteins is the CD44 receptor, which exists in multiple isoforms due to a significant degree of alternative splicing [32]. Glycosylation also broadens CD44 structural diversity and influences receptor activity. Given the polyanionic character of HA, it is at first glance surprising that hydrogen bonding and van der Waals forces dominate the CD44-HA interaction (at least for the murine CD44 receptor), as found by Banerji et al. via co-crystallization [33]. However, the anionic charges are unevenly distributed over the polymer, resulting in a substantial hydrophobic surface along the polyelectrolyte chain. A minimum chain length of six monosaccharides is necessary for monovalent binding of HA to the CD44-receptor [34]. Multivalent binding enhances receptor affinity and can be observed when the polysaccharide chain length exceeds 20 monomers. Mizrahi et al. implemented a model to estimate both, the binding strength between HA and its receptor CD44 and the receptor coverage by HA of different MW [35]. They immobilized a recombi-

Table 1
Estimated CD44-Fc coverage by free HA at different MWs. Assuming a receptor density of 3 receptors per 1000 nm^2 and taking into account the radius of gyration of differently sized HA-chains, the receptor coverage was calculated. With permission taken from [35].

HA MW (kDa)	Radius of gyration (nm)	Area (nm^2)	CD44 coverage (CD44 molecules available per HA)
6.4	4	49	1 (0.16)
31	10	327	1
132	24	1709	5–6
700	66	13,678	44
1500	105	34,365	110