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Equilibrium and release properties of hyaluronic acid–drug complexes

Franco David Battistini, María Eugenia Olivera, Rubén Hilario Manzo *

Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, X5000HUA Córdoba, Argentina
Unidad de Tecnología Farmacéutica (UNITEFA-CONICET), Córdoba, Argentina

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ABSTRACT

With the aim to provide more rational basis about the potentiality of hyaluronic acid (or hyaluronan) as drug carrier a set of ionic complexes of its acid form (HA) and its sodium salt (NaHA) with three model drugs (D) (atenolol, propranolol and lidocaine) were prepared. Besides NaHA subjected to hyalurodinase depolimerization ($NaHA_d$) was also used. Transparent dispersions were obtained. They exhibited negative electrokinetic potential and a high degree of counterionic condensation with affinity constants ($\log K_{cc}$) in the range of 5.8–6.1 for propranolol complexes (pK_a 9.45) and 4.0–4.6 for lidocaine ones (pK_a 7.92).

Delivery rates of D from the complexes were measured in a Franz-type bicompartimental device. Loaded D were slowly released from the three types of complexes, even when a neutral salt was added to the dispersion placed in the donor compartment, revealing the high affinity between the protonated drugs and the ionisable groups of the polymer.

Complex dispersions based on HA or on $NaHA_d$ exhibited lower viscosity than those of NaHA but their complexing ability remained unaltered.

The results reported on equilibrium and release properties of Hyaluronan-model D complexes contribute to expand the use of HA and NaHA as drug carriers for different routes of administration.

1. Introduction

Hyaluronic acid also known as hyaluronan is a naturally occurring polysaccharide belonging to the glycosaminoglycan family, composed by repeated D-glucuronic acid-β (1,3)-N-acetyl-D-glucosamine disaccharide units linked together through β (1,4) glycosidic bonds. Fig. 1 shows the monomeric unit of the acid form (HA). This biopolymer is widely distributed in body tissues being the major constituent of the extracellular matrix of vertebrates. It is involved in many biological processes, such as cellular adhesion, mobility and differentiation (Leach and Schmidt, in press; Delpech et al., 1997; Rooney et al., 1995; Laurent, 1987). Sodium hyaluronate (NaHA) is the high molecular weight fraction of purified natural sodium salt of hyaluronan (European Pharmacopoeia 5.0, 2005). It is widely used as a parenteral and ophthalmic viscoelastic agent, applied in the joints and instilled in the bladder for the treatment of interstitial cystitis (Rah, 2011; Gomis et al., 2009; Iavazzo et al., 2007; Ludwig, 2005; Akira, 2004; Ghosh and Guidolin, 2002).

Hyaluronan has attracted the attention of many scientists to use it in the design of parenteral delivery systems (Hirakura et al., 2010; Lee et al., 2009; Hahn et al., 2005). In relation to this point, the interactions of hyaluronan with CD44 receptors that are overexpressed in several types of cancer (Choi et al., 2012; Slomiany et al., 2009) were recently described.

However, it is well known that hyaluronan exhibits a short biological half-life since it is subjected to degradation by hyaluronidase enzymes (Oh and Kim, 2010; Necas et al., 2008; Fraser and Laurent, 1997; Rooney et al., 1995). This degradative process has been considered as a severe shortcoming that would affect its performance as a drug carrier. In connection with this point, a number of chemical derivatives, in which hyaluronan reactive groups are covalently bonded to other moieties, has been proposed (Young et al., 2012; Akira, 2004; Yuna et al., 2004).

However, hyaluronan bearing a carboxylic group in each glucuronic unit (pK_a 3–4) (Brown and Jones, 2005; Hascall and Lauren, 1997), behaves as an acidic polyelectrolyte (PE) able to form complexes with drugs (D) possessing an appropriate basic group. The strategy relating to the use of different PE–D ionic complexes as drug carriers is currently proposed in the area of drug delivery (Guzmán et al., 2012; Ramirez Rigo et al., 2009; Quinteros et al., 2008; Jiménez Kairuz et al., 2005; Jiménez Kairuz et al., 2003). In this field, Doherty et al. (1995) obtained stable and reversible ionic complexes between lidocaine and medium molecular weight hyaluronan that allowed the prolongation of epidural analgesia when injected into the epidural space in rabbits, although such effect was not observed in dogs (Doherty et al., 1996). Saettone et al. (1991),

* Corresponding author at: Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, X5000HUA Córdoba, Argentina. Tel./fax: +54 351 5353865.
E-mail address: rubmanzo@mail.fcq.unc.edu.ar (R.H. Manzo).

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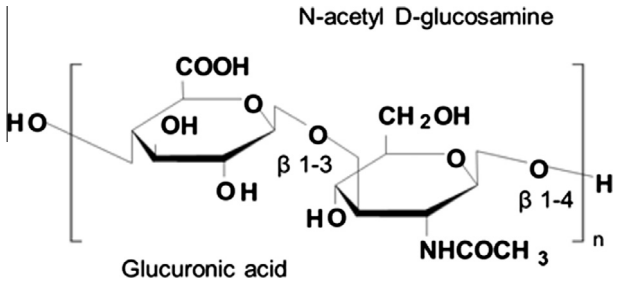

Fig. 1. Structure of the repeating disaccharide unit (N-acetyl-D-glucosamine and D-glucuronic acid) present in hyaluronan.

Table 1
Structural and physicochemical properties of selected drugs.

Drug	MW	Amlne	pK_a	Log	Solubility (mg/mL)
Lidocaine	234.34	Ternary	7.92	2.26	3.98
Atenolol	266.34	Secondary	9.55	0.16	12.8
Propranolol	259.34	Secondary	9.45	3.03	0.12

Saettone et al. (1989) reported some ionic drug complexes for topic ophthalmic formulations. In addition, a patent covering ophthalmic formulations based on drug-hyaluronan salts was also registered (Della Valle et al., 1995). However, at present a detailed knowledge regarding the affinity of protonable D for the carboxylic groups of hyaluronan under different conditions is not available. Therefore, it is of our interest to provide more detailed information concerning this point in order to contribute with more solid bases to evaluate the potentiality of hyaluronan as a drug carrier convenient for being used in specific therapeutic indications.

Then, the main points of concern addressed in this report are:

(i) The ability of commercial NaHA, its hyaluronidase-depolymerized product ($NaHA_d$) and the acid form HA to produce ionic reversible complexes with model basic D according to the following equations:

$$\text{R-COOH} + \text{D} \rightleftarrows \text{R-COO}^- + \text{DH}^+ \rightleftarrows (\text{R-COO}^-\text{DH}^+) \quad (1)$$

$$\text{R-COO}^-\text{Na}^+ + \text{D} + \text{H}_2\text{O} \rightleftarrows \text{R-COO}^- + \text{DH}^+ + \text{OH}^- + \text{Na}^+ \rightleftarrows (\text{R-COO}^-\text{DH}^+) + \text{NaOH} \quad (2)$$

in which R-COOH represents the acid pending groups of hyaluronan, and D and DH^+ represent the unprotonated and protonated species of D.

(ii) How much the equilibrium and release properties of the complexes are affected by environmental conditions such as pH and inorganic ions.

For this purpose, three D that were previously used to describe PE–D interactions (Lidocaine (Li), Atenolol (At) and Propranolol (Pr)) were selected based on their lipophilicity and basicity (Table 1).

2. Materials and methods

2.1. Materials

NaHA, Parafarm®, Bs. As. Argentina, obtained from bacteria's fermentation, (MW = 1655 kDa), was used. Bovine testicular hyaluronidase (HAse), with a specific activity of 801 USP IU/mg, was purchased from Sigma Chemical Co., St Louis, USA. Both, NaHA and HAse, were used without any further purification. At, Li and Pr hydrochloride, all pharmaceutical grade, were obtained from Parafarm®, Bs. As., Argentina. Pr was obtained by neutralization of its hydrochloride salt with 1 N NaOH. The solid product obtained, that was filtered, washed with distilled water and dried in oven to 50 °C to constant weight, melts at 91.21 °C. Phosphate Buffer Saline (PBS, 10 mM, pH 6.80) was prepared according to USP 34-NF 29 (2011). Cyclohexane pharmaceutical grade was purchased from Cicarelli SA (Argentina). All other reactants were of analytical grade. Mili-Q water was used for all the experiments.

2.2. Preparation and characterization of the free acid form HA

HA was obtained after neutralization of NaHA with an ionic exchange resin. Briefly, 100 mL of a 0.5% w/v aqueous dispersion of NaHA (pH = 6.80) was passed through a glass column (4.2 cm diameter and 21 cm high) containing the sulfonic acid resin Amberlite® IR 120 in hydrogen form (Sigma–Aldrich). After that, several 20 mL-water portions were added to the column to get the complete drainage of the HA generated (pH = 2.82). Solid HA was obtained by lyophilization of this solution under a vacuum of 10×10^{-3} mBar after initial freezing with liquid air. Besides, HA was titrated with HCl and NaOH respectively to determine the equivalents of carboxylic groups per gram. They were also subjected to differential scanning potentiometry (DSP) according to Manzo et al. (1991) to assess acidic or basic purity. In order to evaluate the N-acetyl-D-glucosamine, reducing ends of NaHA and HA, 0.1% dispersions of both solids were subjected to the experimental procedure described by Reissig et al. (1995). In this experiment, the reaction between the Ehrlich's reagent (p-Dimethylamino benzaldehyde, DMAB) and N-acetyl-D-glucosamine reducing ends of the hyaluronan chains gave a pink colour (maximum wavelength 585 nm). Briefly, a borate solution was prepared by dissolving 4.94 g boric acid and 1.98 g potassium hydroxide in 100 mL of Milli-Q water. In addition, a 0.1 g/mL DMAB solution was prepared by dissolving 5 g DMAB in 6.25 mL of HCl 12 N and made up to a final volume of 50 mL with glacial acetic acid. The latter solution was 10-fold diluted with glacial acetic acid just before use (and at least 15 min before use). A 200 μL-aliquot of NaHA or HA dispersions was added to 50 μL of the borate solution in a glass tube. The solution in the tube was immediately vortexed, heated in a boiling water bath for exactly 3 min, and then placed in a cold water bath at approximately 10 °C for 1 min. Then, 1.5 mL of the diluted DMAB solution was added to each of these tubes, which were vortexed and placed at 37 °C for exactly 15 min. This was transferred to a plastic cuvette of 1 cm pathlength and immediately scanned by UV–Vis spectroscopy between 400 and 700 nm, using water as reference.

The Fourier Transformed Infrared (FTIR) spectra of 1% solid HA and NaHA dispersed in KBr discs were recorded in a NICOLET FTIR (360 FTIR ESP, Thermo Nicolet, Avatar) spectrometer.

The viscosity of 1% HA and NaHA dispersions as well as that of the HA dispersion added with enough NaOH to neutralize all its carboxylic groups, were measured at 37 °C and 100 RPM in a Haake (Karlsrube, Germany) viscometer VT500 equipped with a software VT500/VT 3.01, and a MV2 sensor.

2.3. Depolymerization of NaHA by HAse

The device shown in Fig. 2 was used to determine the depolymerization of NaHA by action of bovine HAse. Since depolymerization produces a lowering in viscosity, this parameter was selected as a kinetic indicator. Fifteen mL of a 0.66% w/v dispersion of NaHA in PBS was introduced in the reaction vessel provided with a magnetic stirrer and thermostatized at 37 °C. This concentration was selected because its viscosity was appropriate to be followed through time. A solution of HAse (2.5 mg/5 mL) was prepared the day of the experiment by dissolving the enzyme in PBS. An aliquot of 200 μL corresponding to 160.2 IU of HAse, previously incubated for an hour at 37 °C, was added in the NaHA dispersion. A 2 mL pipette provided