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Biodistribution profiling of the chemical modified hyaluronic acid derivatives used for oral delivery system

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ABSTRACT

A series of adipic acid dihydrazide (ADH)-modified hyaluronic acid (HA-ADH) compounds were synthesized and conjugated with QDots (QDots-HA conjugates) to assess the effects of the molecular weight (MW) and extent of chemical modification of HA on its biodistribution. Their physicochemical structures were confirmed by complementary application of GPC, ^{1}H NMR, FTIR, and UV–vis spectroscopic methods. In vivo imaging of QDots-HA conjugates after oral administration was analyzed to investigate their biodistribution in nude mice. Simultaneously, real-time bioimaging was confirmed by an anatomical analysis to investigate the organ-specific accumulation of conjugates. QDot-HA conjugates with a higher MW of HA or high modification presented relatively slow clearance leading to an extension of the retention time for up to 10 days, whereas those with lower MWs of HA or a low modification extent exhibited quick absorption and elimination after oral administration. Taken together, HA derivatives with suitable MWs and chemical modification extents can be used to design new, more-sophisticated, and intelligent HA-based vehicles for oral delivery with diverse characteristics.

1. Introduction

Hyaluronic acid (HA) is a nonsulfated glycosaminoglycan (GAG) that is abundant in connective tissues of vertebrates. It is composed of repeating disaccharide units of D-glucuronic acid and N-acetylglucosamine with a wide molecular weight (MW) range of 10^3–10^7 Da. HA and modified HA are widely used for pharmaceutical and medical applications, such as arthritis treatment [1], ophthalmic surgery [2], drug delivery [3,4], and tissue engineering [5], because of its unique physicochemical properties and various biological functions. In particular, a number of studies reported that HA is capable of being used as a drug delivery agent along various administration routes, including ophthalmic, nasal, pulmonary, parenteral, and topical application [6–8]. The benefits of using HA as a drug delivery vehicle are that it is biocompatible, non-toxic, non-inflammatory, and biodegradable; it can efficiently function as a 'homing device' because the HA receptor cluster determinant 44 (CD44) is overexpressed in many types of tumor cells; it provides protection to its 'cargo'; and it imparts solubility to hydrophobic drugs.

However, the major limitation of HA is its remarkably rapid physiological turnover. The half-life of injected HA in human plasma is 2.5–5.5 min, indicating rapid degradation of HA [9]. Thus, the short half-life of HA in vivo has to be overcome prior to it being used for long-term clinical applications. As is well known, it is possible to prolong the residence time of HA in the body by chemically modifying it. Because the functional groups of HA make it available for chemical conjugation [10,11], a number of strategies for HA modification, mainly through carboxyl and hydroxyl groups, have been developed, including esterification of HA, chemical modification of HA with carbodiimide, and cross-linking of HA with divinyl sulfone (DVS), glycidyl ether, and dialdehyde. These HA derivatives are widely used as carriers for target-specific and long-acting delivery of chemical drugs, such as cisplatin [12], paclitaxel [13], doxorubicin [14], and biopharmaceuticals like protein, peptide, and nucleotide therapeutics [15]. HA derivatives were developed as forms of long-acting conjugates, controlled-release microparticles, and selectively cross-linked hydrogels for drug delivery applications [16–18].

Despite phenomenal advances in the inhalable, injectable, transdermal, nasal and other routes of administration, the unavoidable truth is that oral drug delivery remains well ahead of the pack as the preferred delivery route [19]. Oral delivery continues to be the most popular route of administration due to its versatility, ease of administration and probably most importantly patient compliance. HA is used as a carrier in various oral formulations such as microspheres and complexes to improve the

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solubility and bioavailability of poorly water-soluble drugs [20–22], however several limitations still exist and influence the gastrointestinal absorption of exogenous HA when it is administered orally, such as its relatively high MW and poor liposolubility [23]. This might be the reason that no common oral delivery system for HA is yet on the market. HA derivatives offer potential benefits as a carrier for oral administration; however the detailed in vivo characteristics of these HA derivatives administered orally have not been completely understood yet. Before conclusions can be drawn on possible roles of these formulations in clinical practice, further in vivo studies are required, particularly in the area of uptake mechanisms, transportation through the gut mucosa, and bioavailability. As a consequence, we attempted to provide a comprehensive understanding of the relationship between the physicochemical properties (MW and extent of chemical modification) and pharmacokinetic behaviors of these HA derivatives when administrated orally. Further, to study how this biomolecule specifically interacts with cells in the body when administrated orally and investigate the effect of the MW and chemical modifications of HA in vivo, we took advantage of the fluorescence of quantum dots (QDots) to study the in vivo biodistribution after the oral administration of HA with different MWs and extents of chemical modification.

QDots are semiconductor nanocrystals with a size-tunable photoluminescence, and broad absorption and sharp emission spectra [24]. Recently, QDots with near-infrared emission wavelengths have attracted much interest for in vivo applications [25]. In the study, the QDot-HA conjugates were applied for the purpose of the real-time bioimaging of HA derivatives in nude mice. Finally, applications in oral drug delivery systems of HA derivatives are discussed.

2. Materials and methods

2.1. Materials

Sodium hyaluronate, the sodium salt of HA, with average molecular weights (MW) of 31, 234.4, and 1700 kDa was obtained from Lifecore (Chaska, MN, USA). HA with an average MW of 1000 kDa was obtained from Kibun Food Chemifa (Chuo-ku, Tokyo, Japan). Bovine testicular hyaluronidase, N-hydroxysulfosuccinimide sodium salt (sulfo-NHS sodium salt), adipic acid dihydrazide (ADH), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) hydrochloride, the ingredients of Tris–borate–EDTA (TBE) buffer, and 2-mercaptoethanol were obtained from Sigma–Aldrich (St. Louis, MO, USA). QDots with emission wavelengths of 800 nm (QDot800) were obtained from Invitrogen (Carlsbad, CA, USA). They have carboxyl terminal ligands which are used to conjugate various biomolecules. Nude mice (Balb/c) were purchased from the National Laboratory Animal Center (NLAC, Taipei, Taiwan). All reagents were used without further purification.

2.2. HA enzymatic degradation

An HA phosphate-buffered saline (PBS) solution was placed in a reactor, stirred, and maintained at 37 °C, and the reaction was initiated by adding an appropriate volume of hyaluronidase (HAse) solution ranging 0.002–0.5 mg mL^{-1} previously held at 37 °C with a thermostat. HA solutions with an average MW of 1000 kDa (1000–1400 kDa) and total concentration of 0.4 mg mL^{-1} were mixed with different HAse concentrations of 0, 0.002, 0.005, 0.01, and 0.05 mg mL^{-1} at the following pH values: pH 4, 5, and 6. At each scheduled time point (1, 2, 3, 5, 10, 20, 30, 60, 90, and 120 min), a 200-μL aliquot of the reaction mixture was removed from the reactor, and the enzyme reaction was terminated by placing the mixture into a boiling-water bath for 3 min. After cooling in cold water, every sample was filtered by a syringe filter (pore size: 0.45 μm, Millipore) before measuring their MWs by gel permeation chromatography (GPC). The GPC system consisted of a PN Postnova 1021 solvent delivery system pump, a Jasco As-851 autosampler, a Thermoquest ERC-7512 refractive index (RI) detector, a Tosoh GMPWXL column (7.8 mm × 30 cm; particle size, 13 μm), and a chromatography column thermostat (column oven) temperature set to 25 °C were connected in series. The accuracy results of MW determination by GPC were included in Supplementary data (Fig. S1 and Table S1).

2.3. Preparation and characterization of HA-ADH conjugates and QDot-HA conjugates

Several published procedures [26–29] were implemented to give better control of the degree of chemical modification and to allow for more complete purification of the HA-ADH product. HA-ADH conjugates with various degrees of modification (35%, 50%, and 70% of ADH modification, respectively) were prepared as described elsewhere except a slight modification in the reaction time [30]. Briefly, HA (with MWs of 50, 250, and 1000 kDa) was dissolved in deionized water to give an HA solution of 5 mg mL^{-1}. An appropriate amount of solid ADH (equal to 40-times the molar ratio of HA) was added and mixed for 30 min. The pH of the reaction mixture was adjusted to 4.8 by adding 0.1 N HCl. After that, EDC (at 4-times the molar ratio of HA) was added in solid form. The pH of the reaction mixture was maintained at 4.8 by adding 0.1 N HCl. The reaction was stopped by raising the pH of the reaction mixture to 7.0 with 0.1 N NaOH following the set time points of 5, 10, 15, 30, 60, 120, and 240 min. Dialysis tubing (with a M_w cut-off of 6000–8000) was soaked in water at room temperature for 3–4 h and then rinsed. The reaction mixture was transferred to prewashed dialysis tubing and exhaustively dialyzed (60 h against 100 mM NaCl), followed by dialysis against alternating solutions of 1:3 EtOH–H_2O (v/v) and pure H_2O. The solution was then centrifuged, and the supernatant was lyophilized. The chemical structures of HA-ADH conjugates were then confirmed by Fourier-transform infrared (FTIR) spectroscopy (Perkin Elmer FTIR-ATR 100 series, USA) and an ultraviolet-visible (UV-vis) spectrophotometer (Jasco UV-vis V-550, Japan), while the degree of substitution by ADH was assessed by ^{1}H nuclear magnetic resonance (NMR) spectroscopy (NMR advance DRX 500, Bruker, Italy).

QDot-HA conjugates were synthesized as previously reported from Kim et al. [31]. HA-ADH with different MWs of HA and various degrees of modification were then conjugated to the QDots by a coupling reaction between the —NH_2 of HA-ADH and the sulfo-NHS of the QDots. The recovered QDot-HA conjugates were then stored in a refrigerator for further use. Furthermore, the physicochemical properties of the QDot-HA conjugates were characterized and confirmed by UV–vis, fluorescence spectrometer (Jasco FP-6600, Japan) and GPC.

2.4. In vivo real-time imaging of QDot and QDot-HA conjugates

Male nude mice (BALB/cAnN.Cg-Foxn1nu/CrlNarl), at 6–8 weeks old, were purchased from the National Laboratory Animal Center (Taipei, Taiwan). QDot-HA conjugates (150 μL, ca. 0.1 nmol) with different MWs of HA and various ADH contents were orally administered to nude mice at a concentration of 10 mL kg^{-1}. Nude mice were starved for 12 h before the oral-administration experiments. The body was bioimaged in real time after oral administration to nude mice. The fluorescence of the administrated QDot-HA conjugates was captured with a luminescent image analyzer (XENOGEN Living Image®, USA) post-injection, and at the following time points of 6 min, 30 min, 1 h, 12 h, and 1, 3, 7, and 10 days. The relative