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Targeted systemic mesenchymal stem cell delivery using hyaluronate – wheat germ agglutinin conjugate

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ABSTRACT

A variety of receptors for hyaluronate (HA), a natural linear polysaccharide, were found in the body, which have been exploited as target sites for HA-based drug delivery systems. In this work, mesenchymal stem cells (MSCs) were surface-modified with HA - wheat germ agglutinin (WGA) conjugate for targeted systemic delivery of MSCs to the liver. WGA was conjugated to HA by coupling reaction between aldehyde-modified HA and amine group of WGA. The conjugation of WGA to HA was corroborated by gel permeation chromatography (GPC) and the successful surface modification of MSCs with HA-WGA conjugate was confirmed by confocal microscopy. The synthesized HA-WGA conjugate could be incorporated onto the cellular membrane by agglutinating the cell-associated carbohydrates. Fluorescent imaging for *in vivo* biodistribution visualized the targeted delivery of the HA-WGA/MSC complex to the liver after intravenous injection. This new strategy for targeted delivery of MSCs using HA-WGA conjugate might be successfully exploited for various regenerative medicines including cell therapy.

1. Introduction

Cell therapy has a great potential to treat various diseases or to replace damaged tissues. Recently, mesenchymal stem cells (MSCs) have been widely investigated for cell therapy because they can differentiate into diverse lineages, secrete a variety of growth factors and cytokines, and carry cargos as vehicles for gene and drug delivery [1,2]. To improve the efficacy of MSC therapy, the spatiotemporal control of MSCs becomes very important with consideration of the origin, the passage number, and the injected number of MSCs. MSCs are generally injected into the body by either local or systemic delivery. Although the local injection of MSCs has the advantage of direct MSC delivery in a quantity, the local injection into internal organs is relatively invasive due to tissue damages resulting from the surgical operation [3]. In contrast, the systemic injection has the advantage of relative noninvasiveness, but suffers from non-specific delivery and low efficient delivery of MSCs to the target site. Generally, intravenous injection of MSCs results in the initial entrapment mainly in lungs, causing a common respiratory failure in clinical practice [4,5]. Although MSCs can be redistributed from lungs to other sites, migration of MSCs to the target site depends on the innate characteristics of MSCs such as the homing ligands of MSCs [6,7]. MSCs express only a small amount of key homing receptors and lose their homing ability during *in vitro* culture expansion [8,9]. Accordingly, it would be greatly beneficial to systemically deliver MSCs to a target region in the body after intravenous injection [10].

Hyaluronate (HA) is a biocompatible, biodegradable, and non-toxic polysaccharide which has been extensively investigated for target-specific drug delivery to several receptors in the body [11,12]. Especially, because HA receptors are highly expressed in the liver including HA receptor for endocytosis (HARE) [13] and cluster determinant 44 (CD44) [14,15], HA-based drug delivery systems frequently target the liver for the treatment of liver diseases. In our previous works, real-time bioimaging for the target-specific delivery of HA derivatives to the liver was demonstrated using quantum dots [16], carbon dots [17], and zwitter ionic dyes [18]. Furthermore, HA-interferon alpha conjugate was successfully applied for the treatment of hepatitits C virus infection [19], HA-tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) conjugate for liver fibrosis [20], and HA-Flt1 peptide conjugate for liver cancer [21]. All these results reflect the feasibility of HA derivatives for target-specific delivery of therapeutic drugs and cells to the liver.

In this study, we investigated the cellular surface modification to change the functions and properties of MSCs such as adhesion,

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migration, tissue homing, and cell-cell interactions [22–24]. For the target-specific systemic delivery of MSCs to the liver, we synthesized HA - wheat germ agglutinin (WGA) conjugate by the coupling reaction of HA-aldehyde to the amine group of WGA. Since WGA is a protein which can specifically recognize and agglutinate cell-associated carbohydrates such as sialic acid and N-acetyl-D-glucosamine [25–28], HA-WGA conjugate was thought to modify the surface of MSCs and deliver them systemically to the liver. After characterization of HA-WGA conjugate by gel permeation chromatography (GPC) and circular dichroism (CD), the cytotoxicity of HA-WGA conjugate and the incorporation of HA-WGA conjugate onto the cellular membrane of MSCs were assessed by MTT assay and confocal microscopy, respectively. Finally, we visualized *in vivo* liver-targeted delivery of MSCs surface-modified with HA-WGA conjugate (HA-WGA/MSC) using an IVIS imaging system and a fluorescence microscopy.

2. Materials and methods

2.1. Materials

Sodium hyaluronate (HA) with a molecular weight (MW) of 100 kDa was purchased from Lifecore Biomedical (Chaska, MN). Sodium periodate, sodium cyanoborohydride, wheat germ agglutinin (WGA), ethyl carbazate, fluorescein isothiocyanate (FITC), carboxyfluorescein succinimidyl ester (CFSE), N-hydroxysulfosuccinimide (sulfo-NHS), and dimethylsulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO). The Bradford protein assay kit was purchased from Thermo scientific (Rockford, IL). HiLyte Fluor™ 647 (HiLyte647) NHS ester dye, HiLyte647 amine and 4′,6-diamidino-2-phenylindole (DAPI) were obtained from Anaspec Inc. (Fremont, CA). The tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) enzyme-linked immunosorbent assay (ELISA) kit was purchased from R&D system (Minneapolis, MN). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Promega (Madison, WI). A 24-well transwell insert was purchased from Milipore (Bedford, MA) and phosphate buffered saline (PBS, pH 7.4) from Tech & Innovation (Seoul, Korea). 5-Aminofluorescein (AF) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) hydrochloride were purchased from Tokyo Chemical Industry (Tokyo, Japan). The EZ-cytox cell viability assay kit was provided from Daeil Labservice (Seoul, Korea). High-glucose Dulbecco's modified Eagle's medium (HDMEM), low-glucose Dulbecco's modified Eagle's medium (LDMEM), fetal bovine serum (FBS), and antibiotics were obtained from Gibco (Grand Island, NY). Human MSCs (hMSCs) were purchased from Neuromics Co. (Edina, MN) and HepG2 cells from ATCC (Manassass, VA).

2.2. Synthesis of HA-WGA conjugate

Aldehyde-modified HA (HA-aldehyde) was prepared as reported elsewhere [19]. To synthesize HA-WGA conjugate, HA-aldehyde with an aldehyde content of 10 mol% was dissolved in sodium acetate buffer (pH 5.5) at a concentration of 5 mg/mL. The number of WGA molecules per single HA chain in the feed was adjusted to be four. The conjugation reaction was performed at room temperature for 24 h with stirring. Five-molar excess of sodium cyanoborohydride to aldehyde groups was added to the reaction solution to reduce hydrazine linkage. The unreacted aldehyde groups in HA-WGA conjugate were blocked with 5-M excess of ethyl carbazate in the presence of sodium cyanoborohydride at room temperature for another 24 h. Then, the HA-WGA conjugate was purified using a dialysis tube (MWCO = 50 kDa) against a large excess of PBS.

2.3. Characterization of HA-WGA conjugate

The successful synthesis and concentration of HA-WGA conjugate were assessed by GPC and Bradford assay. The GPC analysis was performed to check the retention time before and after conjugation of WGA to HA using the following systems: Waters 717 plus autosampler, Waters 1525 binary HPLC pump, Waters 2487 dual wavelength absorbance detector, Ultrahydrogel™ 1000 connected with Ultrahydrogel™ 500 column. The eluent was PBS and the flow rate was 0.4 mL/min. The detection wavelengths were 210 nm for HA and 280 nm for WGA. The Bradford assay was conducted according to the manufacturer's instructions.

The secondary structure of HA-WGA conjugate was analyzed by CD spectroscopy. The CD spectra for WGA and HA-WGA conjugate in PBS were obtained using a UV spectrophotometer (JASCO J-715, Essex, UK) under N_2 atmosphere. A quartz cuvette with a path length of 2 mm was used and the data were acquired at 0.2 mm intervals with a response time of 1 s. Each spectrum was subtracted by the spectrum of PBS and the residual ellipticity was calculated by averaging the results of three scans.

2.4. Cytotoxicity of HA-WGA conjugate

Rat MSCs (rMSCs) were prepared as previously reported elsewhere [29]. Before the surface modification of MSCs, cytocompatibility of HA-WGA conjugate on MSCs was assessed using an MTT assay. Briefly, MSCs were seeded at 1×10^4 cells per well in 96-well plates and incubated at 37 °C and 5% CO_2 for 24 h, then treated with HA-WGA conjugate at different concentrations of WGA. After 24 h incubation, MSCs were washed with PBS twice, and then the medium in each well was replaced with a medium containing the MTT reagent. After further incubation until purple precipitates were visible, each well was aspirated to remove the medium and filled with DMSO to dissolve the formazan crystal. The absorbance at 540 nm was measured with a microplate reader (EMax, Molecular Devices, CA). Cell viability (%) was calculated using Cell viability = $[A_{540\ (sample)}/A_{540\ (control)}] \times 100$, where $A_{540\ (sample)}$ is the optical density from wells treated with WGA or HA-WGA conjugate and $A_{540\ (control)}$ is that from wells treated with PBS.

2.5. Surface modification of MSCs with HA-WGA conjugate

Cultured MSCs were trypsinized, washed, and resuspended at 1×10^6 cells/mL in PBS. HA-WGA conjugate at 10 μg/mL of WGA was added to MSCs in suspension, and incubated in an ice bath for 10 min with mild mixing. To remove the unbound HA-WGA conjugate to MSCs, cell suspension was washed with PBS and collected by centrifugation (1000 × g, 3 min at 4 °C). After surface modification with HA-WGA conjugate, HA-WGA/hMSC was resuspended in the cell culture medium on 96-well plates (1×10^4 cells per well). At the predetermined time, cell viability of HA-WGA/hMSC was measured using an EZ-cytox cell viability assay kit according to the manufacturer's instructions. Zeta potentials were analyzed using a Zetasizer Nano (Malvern Instruments, UK) to assess the change of surface charge after the surface modification.

2.6. Labeling HA-WGA conjugate with fluorescent dyes

HA-WGA conjugate was labeled with fluorescent dye (FITC or HiLyte647 NHS ester). The dye solution in DMSO was added to the HA-WGA conjugate solution at a 5-M ratio to protein molecule. The conjugation reaction was performed at room temperature overnight with mild stirring. The dye-labeled HA-WGA conjugate was purified using desalting columns. WGA-FITC was prepared using the same method. For comparison, HA-FITC was prepared as a