Biomaterials 32 (2011) 4118–4129

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

N-acetylgalactosamine-functionalized dendrimers as hepatic cancer cell-targeted carriers

Scott H. Medina [a], Venkatesh Tekumalla [a], Maxim V. Chevliakov [a], Donna S. Shewach [b], William D. Ensminger [c], Mohamed E.H. El-Sayed [a,d,*]

[a] University of Michigan, Department of Biomedical Engineering, Cellular Engineering and Nano-Therapeutics Laboratory, 1101 Beal Avenue, Ann Arbor, MI 48109, USA
[b] University of Michigan, Department of Pharmacology, 1150 W. Medical Center Drive, Ann Arbor, MI 48109, USA
[c] University of Michigan, Department of Internal Medicine, 1150 W. Medical Center Drive, Ann Arbor, MI 48109, USA
[d] University of Michigan, Macromolecular Science and Engineering Program, Ann Arbor, MI 48109, USA

ARTICLE INFO

Article history:
Received 15 November 2010
Accepted 27 November 2010
Available online 22 March 2011

Keywords:
Nanoparticle
Dendrimers
Drug delivery
Flow cytometry
Hepatocyte

ABSTRACT

There is an urgent need for novel polymeric carriers that can selectively deliver a large dose of chemotherapeutic agents into hepatic cancer cells to achieve high therapeutic activity with minimal systemic side effects. PAMAM dendrimers are characterized by a unique branching architecture and a large number of chemical surface groups suitable for coupling of chemotherapeutic agents. In this article, we report the coupling of *N*-acetylgalactosamine (NAcGal) to generation 5 (G5) of poly(amidoamine) (PAMAM-NH_2) dendrimers via peptide and thiourea linkages to prepare NAcGal-targeted carriers used for targeted delivery of chemotherapeutic agents into hepatic cancer cells. We describe the uptake of NAcGal-targeted and non-targeted G5 dendrimers into hepatic cancer cells (HepG2) as a function of G5 concentration and incubation time. We examine the contribution of the asialoglycoprotein receptor (ASGPR) to the internalization of NAcGal-targeted dendrimers into hepatic cancer cells through a competitive inhibition assay. Our results show that uptake of NAcGal-targeted G5 dendrimers into hepatic cancer cells occurs via ASGPR-mediated endocytosis. Internalization of these targeted carriers increased with the increase in G5 concentration and incubation time following Michaelis–Menten kinetics characteristic of receptor-mediated endocytosis. These results collectively indicate that G5-NAcGal conjugates function as targeted carriers for selective delivery of chemotherapeutic agents into hepatic cancer cells.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Tomalia and co-workers first reported the synthesis of poly(amidoamine) (PAMAM) dendrimers as a new class of branched, water-soluble polymers in 1985 [1]. PAMAM dendrimers are characterized by a unique tree-like branching architecture and exhibit a characteristic increase in size, molecular weight, and number of surface functional groups with the increase in generation number [2]. Aqueous solubility, monodispersity, and large number of surface groups of PAMAM dendrimers available for conjugation of therapeutic molecules, targeting ligands, and imaging agents make these polymers ideal carriers for both diagnostic and therapeutic applications [2–6]. For example, PAMAM dendrimers have been used as gene [7–9] and anticancer drug carriers [2,3,10] that may display an antibody for cell or tissue targeting [11,12].

In this article, we report the synthesis of PAMAM-sugar conjugates that can target hepatic cancer cells for selective drug delivery. We selected generation 5 (G5) of PAMAM-NH_2 dendrimers as a carrier due to the large number of primary amine surface groups that can be used for attachment of drug molecules, imaging agents, and targeting ligands. To develop a carrier that can selectively target hepatic cancer, we utilized G5 dendrimers with a diaminobutane (DAB) core to construct the *N*-acetylgalactosamine-functionalized carriers due to their intrinsic ability to accumulate in the liver compared to dendrimers with ethylenediamine cores (EDA) [5,6]. In addition, G5 dendrimers with a DAB core proved to preferentially extravasate across the leaky tumor vasculature while exhibiting insignificant extravasation across normal blood vessels [13], which allows effective *in vivo* targeting of these carriers to tumor tissue [12,14–17]. We envision that conjugation of *N*-acetylgalactosamine (NAcGal) molecules to G5 dendrimers will provide an additional

* Corresponding author. University of Michigan, Department of Biomedical Engineering, 1101 Beal Avenue, Lurie Biomedical Engineering Building, Room 2150, Ann Arbor, MI 48109, USA. Tel.: +1 (734) 615 9404; fax: +1 (734) 647 4834.
E-mail address: meslayed@umich.edu (M.E.H. El-Sayed).
URL: http://www.bme.umich.edu/centlab.php

0142-9612/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2010.11.068

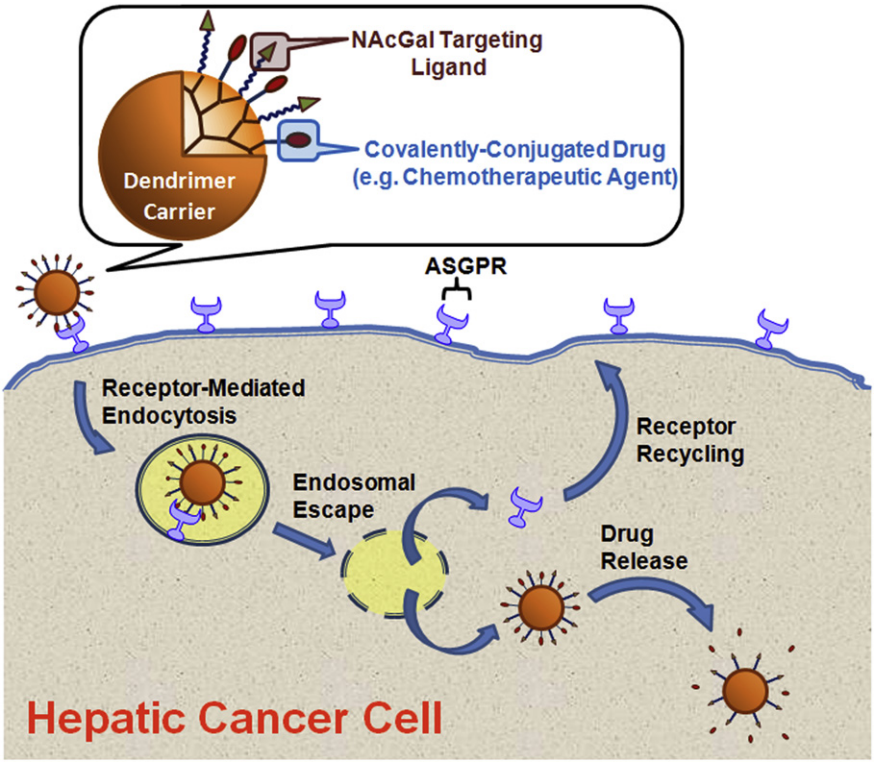

Fig. 1. A schematic drawing showing the composition of a drug-loaded G5-NAcGal conjugate binding to the ASGPR expressed on the surface of hepatic cancer cells (e.g. HepG2), which triggers receptor-mediated endocytosis of these G5-NAcGal conjugates followed by endosomal escape and release of the therapeutic cargo into the cytoplasm while the ASGPR recycles back to the cell surface.

strategy to increase their accumulation and retention in hepatic tumor tissue and warrant their use as carriers for targeted delivery of chemotherapeutic agents.

We report the conjugation of *N*-acetylgalactosamine (NAcGal) sugar molecules to the primary amine surface groups of G5-$(NH_2)_{128}$ dendrimers via peptide and thiourea linkages to prepare G5-NAcGal conjugates with various sugar density. These G5-NAcGal conjugates are designed to achieve selective binding to the asialoglycoprotein receptor (ASGPR) that is highly expressed on the surface of hepatic cancer cells [18], which will trigger their receptor-mediated endocytosis into hepatic cancer cells (Fig. 1). We evaluated the effect of surface charge, concentration, incubation time, number of conjugated NAcGal molecules, and linkage chemistry on the uptake of G5-NAcGal conjugates into human hepatic cancer cells (HepG2). Selectivity of G5-NAcGal conjugates toward hepatic cancer cells and the contribution of the ASGPR to conjugate's internalization was also evaluated using a competitive inhibition assay and assessing conjugates' uptake into MCF-7 breast cancer cells, which lack the ASGPR. MCF-7 breast cancer cells were selected for this comparison due to their high endocytic capacity and their reported use as a control cell line to test selectivity of galactosylated carriers toward the ASGPR [19].

2. Materials and methods

2.1. Materials

G5-$(NH_2)_{128}$ PAMAM dendrimers with DAB core, *N*-acetylgalactosamine, fluorescein isothiocyanate (Fl), and bovine insulin were purchased from Sigma-Aldrich Inc. (St. Louis, MO). Minimum essential medium (MEM), OPTI-MEM reduced serum medium, fetal bovine serum (FBS), 0.25% trypsin/0.20% ethylene diamine teraacetic acid (EDTA), phosphate buffered saline (PBS), penicillin/streptomycin/amphotericin, sodium pyruvate, and non-essential amino acid solutions were purchased from Invitrogen Corporation (Carlsbad, CA). Cytotoxicity Detection Kit (Lactate Dehydrogenase; LDH) was purchased from Roche Diagnostics Corporation (Indianapolis, IN). T-75 flasks, Costar 24-well plates, and cell culture supplies were purchased from Corning Inc. (Corning, NY). HepG2 and MCF-7 cells were generous gifts from Dr. Donna Shewach and Dr. Sofia Merajver, respectively.

2.2. Synthesis of fluorescently-labeled G5-NAcGal conjugates via peptide linkages

2.2.1. Synthesis of 3-(carbo-t-butoxymethyl)-2-(acetylamino)-2-deoxy-D-galactopyranoside (*1*)

NAcGal (486 mg, 2.2 mmol) was dissolved in 10 ml of dimethylformamide (DMF) and NaH (88 mg, 2.2 mmol) was added as a solid followed by the addition of tert-butyl bromoacetate (429.1 mg, 2.2 mmol) in 2 ml of DMF. After stirring at room temperature for 72 h, DMF was removed under reduced pressure and the reaction mixture was purified on a silica gel column using a consecutive eluent system of CH_2Cl_2 (100%), CH_2Cl_2:methanol (15:1), CH_2Cl_2:methanol (10:1) and finally CH_2Cl_2:methanol (8:1) to produce 400 mg (54% yield) of product **1** as a white solid. ^{1}H NMR of compound **1** in CD_3OD (400 MHz Varian, Palo Alto, CA) shows δ 4.92 (d, $J = 4.8$ Hz, 1H), 4.36 (dd, $J_1 = 9.2$ Hz, $J_2 = 4.8$ Hz, 1H), 4.26 (dd, $J_1 = 9.2$ Hz, $J_2 = 7.2$ Hz, 1H), 4.15 (s, 2H), 3.82 (dd, $J_1 = 7.6$ Hz, $J_2 = 3.6$ Hz, 1H), 3.62–3.52 (m, 3H), 3.29 (m, 1H), 2.00 (s, 3H), 1.45 (s, 9H); ESI Mass of compound **1** is 358 $[M + Na]^+$.

2.2.2. Synthesis of 3-(carbo-t-butoxymethyl)-2-(acetylamino)-2-deoxy-D-galactopyranoside-3,4,6-triacetate (*2*)

Excess of pyridine (0.145 ml, 1.79 mmol) and acetic anhydride (0.34 ml, 3.58 mmol) were added to compound **1** (67 mg, 0.179 mmol) dissolved in 2 ml of anhydrous dichloromethane and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was diluted with 50 ml CH_2Cl_2, washed twice with 20 ml of 0.1 N HCl followed by an additional wash with 20 ml DI water. The organic layer was dried over $MgSO_4$ and concentrated providing 57 mg (69% yield) of product **2** as a colorless oil, which was used as the crude product in the next step without any further purification. ^{1}H NMR of compound **2** in $CDCl_3$ (500 MHz Varian, Palo Alto, CA) shows δ 6.48 (d, $J = 9.0$ Hz, 1H), 5.36 (t, $J = 7.0$ Hz, 1H), 5.17 (q, $J = 5.5$ Hz, 1H), 4.97 (m, 1H), 4.66 (m, 1H), 4.23 (dd, $J_1 = 12.0$ Hz, $J_2 = 4.5$ Hz, 1H), 4.17 (m, 2H), 4.10 (dd, $J_1 = 12.0$ Hz, $J_2 = 6.0$ Hz, 1H), 3.98 (d, $J = 16.5$ Hz, 1H), 2.08 (s, 3H), 2,04 (s, 3H), 2.03 (s, 3H), 1.97 (s, 3H), 1.46 (s, 9H); ESI Mass of compound **2** is 462 $[M + H]^+$.

2.2.3. Synthesis of 3-(carboxymethyl)-2-(acetylamino)-2-deoxy-D-galactopyranoside-3,4,6-triacetate (*3*)

Trifluoroacetic acid (1 ml) was added to a solution of compound **2** (55 mg, 0.119 mmol) dissolved in 2 ml of CH_2Cl_2 and the reaction mixture was stirred at room

Download English Version:

https://daneshyari.com/en/article/7143

Download Persian Version:

https://daneshyari.com/article/7143

Daneshyari.com