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Galactosylated alginate-curcumin micelles for enhanced delivery of curcumin to hepatocytes

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ABSTRACT

Galactosylated alginate-curcumin conjugate ($LANH_2$-Alg Ald-Cur) is synthesized for targeted delivery of curcumin to hepatocytes exploiting asialoglycoprotein receptor (ASGPR) on hepatocytes. The synthetic procedure includes oxidation of alginate (Alg), modification of lactobionic acid (LA), grafting of targeting group (modified lactobinic acid, $LANH_2$) and conjugation of curcumin to alginate. Alginate-curcumin conjugate (Alg-Cur) without targeting group is also prepared for the comparison of properties. $LANH_2$-Alg Ald-Cur self assembles to micelle with diameter of 235 ± 5 nm and zeta potential of -29 mV in water. Cytotoxicity analysis demonstrates enhanced toxicity of $LANH_2$-Alg Ald-Cur over Alg-Cur on HepG2 cells. Cellular uptake studies confirm that $LANH_2$-Alg Ald-Cur can selectively recognize HepG2 cells and shows higher internalization than Alg-Cur conjugate. Results indicate that $LANH_2$-Alg Ald-Cur conjugate micelles are suitable candidates for targeted delivery of curcumin to HepG2 cells.

1. Introduction

Cancer therapy remains challenging, even with the development of large number of anticancer drugs. Delivering therapeutics to the entire region of tumor in an appropriate concentration is an intricate task and is not easily achievable with conventional cancer therapy techniques. Anticancer drugs used in chemotherapy often requires modification to increase solubility, circulation time and alteration to reduce adverse side effects and nonspecific activity [1]. Among synthetic and natural anticancer drugs, curcumin has drawn special attention since it can suppress cell proliferation by inducing cell cycle arrest and can cause apoptosis in various cancer cells [2–4]. Curcumin is a yellow spice derived from the roots of the plant *Curcuma longa*. Curcumin exhibits a wide range of pharmacological effects such as anti-inflammatory, anticancer, and anti-angiogenic properties [5–7]. Therapeutic activity of curcumin against hepatocarcinoma cells have been studied and proved to be promising [8–10]. Despite its therapeutic efficacy and safety, curcumin has not been widely utilized for treatment. Poor absorption, rapid metabolism and fast elimination attenuate the bioavailability of curcumin. To improve the therapeutic efficacy of curcumin and to overcome the aforementioned shortcomings, effective drug delivery systems have been developed. Polymeric micelles [11], liposomes [12], nanogels [13], and microspheres [14] are important among these drug delivery systems.

Nanosized polymer drug conjugates based on biocompatible and water soluble polymers like polysaccharides have received tremendous interest for targeted cancer therapy [15]. Polymeric drug conjugates improve solubility of hydrophobic drugs, protect the drug from degradation and increase their bioavailability [16]. Conjugation of tumor specific targeting units to nanoparticles appears to be a promising strategy to attack cancer cells selectively. Targeted conjugates show enhanced efficacy compared to the non targeted polymer drug conjugates [17–21].

Sodium alginate is selected as starting polymer for the present work considering its biocompatibility, biodegradability and presence of large number of functional groups for curcumin conjugation [22]. Alginate is a polyanionic polysaccharide composed of β-D-mannuronic acid and α-L-guluronicacid units and is used in many biomedical applications. Dey and Sreenivasan have developed alginate-curcumin conjugate for enhancing solubility and stability of curcumin [23]. The conjugate is found to be cytotoxic toward L-929 cells. The aim of the present work is to check the suitability of the alginate-curcumin conjugate for the enhanced delivery of curcumin to hepatocytes by attaching a galactose moiety on alginate. Hepatocytes bear asialoglycoprotein receptor (ASGPR), which

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has been validated as a potential target for selective drug delivery to the liver cells. Galactosyl or lactosyl moieties are attached to hepatocyte targeted delivery systems since asialoglycoprotein receptors on hepatoma cells can specifically bind with ligands containing β-D-galactose and *N*-acetylgalactosamine residues [24–26]. Galactosylated alginate is also proved to be suitable for scaffold preparation for hepatocyte attachment [27,28].

In this study, a polymer–drug conjugate is developed from alginate and curcumin with galactose moiety on its surface and its efficiency to carry curcumin to hepatocarcinoma cells is evaluated.

2. Materials and methods

2.1. Materials

Sodium alginate (Medium Viscosity Grade, viscosity of 2% solution: 2000 cps at 25 °C and number average molecular weight by GPC analysis is 494,134 Da), sodium metaperiodate, dimethylaminopyridine (DMAP), *N,N′*-dicyclohexylcarbodiimide (DCC), Minimum Essential Medium (MEM), glutamine, sodium bicarbonate, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), propidium iodide (PI) and Trypsin-EDTA were obtained from Sigma–Aldrich, Saint Louis, USA and Fetal Bovine Serum (FBS) was procured from Invitrogen, USA. Sodium chloride, sodium tetra borate (borax), disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium carbonate, sodium hydroxide, isopropanol and dimethyl sulfoxide (DMSO) were obtained from Merck (Mumbai, India). Curcumin was obtained as a gift from Synthite Industries Ltd., Kolenchery, Kerala, India. Dialysis tubing (6000–8000, 3500 MWCO) was procured from Spectrum Laboratories Inc., CA, USA.

2.2. Periodate oxidation of sodium alginate

Alginate aldehyde (Alg Ald) was prepared by a previously reported procedure [29]. Sodium alginate (10 g) was dissolved in ethanol–water mixture (1:1, v/v). Sodium periodate (6.05 g, required for 50% oxidation) was added to the reaction mixture and stirred at 20 °C for 6 h in dark. Purification was done by dialysis using a dialysis tube of MWCO 3500 for three days against distilled water. Then the dialysate was frozen and lyophilized. Alginate aldehyde (Alg Ald) was characterized by FT-IR spectroscopy and aldehyde content was evaluated by titrimetry [30,31]. Alginate aldehyde (0.1 g) was dissolved in 25 ml, 0.25 N aqueous solution of hydroxylammonium chloride. Liberated hydrochloric acid resulting from the reaction of aldehyde groups with hydroxylamine hydrochloride was titrated against 0.1 N NaOH using methyl orange (0.05% solution, w/v) as indicator. The color change from red to yellow was taken as endpoint. The number of moles of NaOH consumed is equivalent to the number of moles of aldehyde present in the sample.

2.3. Modification of lactobionic acid

Lactobionic acid was modified with ethylenediamine to introduce amino groups by a reported procedure [27,32]. Lactobionic acid (5 g, 0.0129 mol) was refluxed with 30 fold excess of ethylenediamine (26.02 ml, 0.389 mol) dissolved in anhydrous DMSO at 70 °C for 2 h. The monoamine terminated lactobionic lactone ($LANH_2$) was precipitated with chloroform and the obtained precipitate was dried under reduced pressure.

2.4. Conjugation of $LANH_2$ to Alg Ald

$LANH_2$-Alg Ald was prepared by conjugation of $LANH_2$ to Alg Ald by Schiff's base reaction between the aldehyde groups of Alg Ald and the amino groups of $LANH_2$ under basic conditions. Briefly, 0.5 g of Alg Ald was dissolved in 5 ml of 0.1 M borax. To this solution,1.2 g of $LANH_2$ dissolved in water (5 ml) was added and stirred magnetically for 4 h. The solution was dialyzed to remove the unreacted $LANH_2$ using dialysis tube (MWCO 3500) for one day. The purified dialysate was freeze dried.

2.5. Preparation of $LANH_2$-Alg Ald-Cur

Curcumin was conjugated to $LANH_2$-Alg Ald by DCC/DMAP coupling reaction. Briefly, 0.5 g of $LANH_2$-Alg Ald was dissolved in anhydrous DMSO. To this, DCC (0.410 g, 0.0019 mol) and DMAP (0.180 g, 0.0014 mol) were added and stirred under nitrogen at room temperature for 1 h to activate the carboxylic acid groups in Alg Ald. Curcumin (0.15 g, 0.0004 mol) was dissolved in anhydrous DMSO and added to the reaction mixture. The reaction mixture was stirred at 60 °C for 6 h. The unreacted curcumin was removed by dialysis (MWCO 3500) in DMSO for one day followed by dialysis in distilled water for 3 days. Purified dialysate was freeze dried and kept under refrigeration. Alg-Cur conjugate was prepared by a previously reported procedure [23].

2.6. Characterization

FT-IR spectra were recorded on PerkinElmer FT-IR spectrometer (UATR by transmission) in the range of 4000–400 cm^{-1} and 32 scans per sample. CHN elemental analysis (PerkinElmer 2400 Series II CHNS/O Elemental Analyzer) was performed to confirm conjugation of Alg to $LANH_2$. Carbon, hydrogen and nitrogen contents were measured for Alg Ald and $LANH_2$-Alg Ald. NMR spectra were recorded in DMSO-d_6 using 500 MHz spectrometer (Bruker Avance DRX 500). Alg-Cur and $LANH_2$-Alg Ald-Cur conjugates self assemble to micelles when they are dispersed in water. Hydrodynamic diameter and zeta potential were measured by particle size analyzer (Malvern Zeta sizer) with a He–Ne laser beam at a wavelength of 633.8 nm. The size measurement was carried out at a concentration of 1 mg/ml of both the conjugates in deionized water at 25 °C. Scanning electron microscopy (FEI Quanta FEG 200HR Scanning Electron Microscope) was performed to analyze the morphology and size of the conjugates. For SEM, a drop of the conjugate (1 mg/ml) was placed on a glass slide and allowed to air-dry at ambient temperature. The samples were sputter coated with gold and were observed under SEM.

2.7. Estimation of curcumin conjugated to $LANH_2$-Alg Ald-Cur

Curcumin conjugated to $LANH_2$-Alg Ald-Cur and Alg-Cur was estimated by plotting a standard curve prepared using free curcumin. A stock solution of curcumin was prepared in DMSO–water mixture (1:1, v/v). From this stock solution (1 mg/1 ml), different concentrations of curcumin ranging from 0.002 mg/ml to 1 mg/ml were prepared. Concentration of curcumin in the conjugate was estimated from the standard curve by measuring the absorption intensity at 434 nm.

2.8. Determination of critical micelle concentration

The critical micelle concentration (CMC) of $LANH_2$-Alg Ald-Cur and Alg-Cur conjugates were determined by fluorescence spectroscopy using pyrene as the fluorescence probe [33]. Briefly, 5 μl of pyrene solution (6.0×10^{-5} M) in acetone was added to a series of vials followed by evaporation to remove the acetone. Aqueous solutions of both the conjugates with concentrations ranging from 0.001 μg/ml to 0.5 μg/ml were added to each vial and solicited in an ultrasonic bath for 40 min to equilibrate pyrene and the conjugates, and then were left undisturbed overnight at room temperature.