Gum arabic-curcumin conjugate micelles with enhanced loading for curcumin delivery to hepatocarcinoma cells

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Curcumin is conjugated to gum arabic, a highly water soluble polysaccharide to enhance the solubility and stability of curcumin. Conjugation of curcumin to gum arabic is confirmed by $^1$H NMR, fluorescence and UV spectroscopy studies. The conjugate self assembles to spherical nano-micelles (270 ± 5 nm) spontaneously, when dispersed in aqueous medium. Spherical morphology of the self assembled conjugate is evidenced by field emission scanning electron microscopy and transmission electron microscopy. The self assembly of the amphiphilic conjugate into micelle in aqueous medium significantly enhances the solubility (900 fold of that of free curcumin) and stability of curcumin in physiological pH. The anticancer activity of the conjugate micelles is found to be higher in human hepatocellular carcinoma (HepG2) cells than in human breast carcinoma (MCF-7) cells. The conjugate exhibits enhanced accumulation and toxicity in HepG2 cells due to the targeting efficiency of the galactose groups present in gum arabic.

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1. Introduction

Curcumin, a hydrophobic polyphenol obtained from Curcuma longa plant, possessing a variety of therapeutic properties, has been extensively used for centuries in Ayurvedic medicinal formulations (Aggarwal, Surh, & Shishodia, 2007; Ammon & Wahl, 1991; Hatcher, Planalp, Cho, Torti, & Torti, 2008; Maheshwari, Singh, Gaddipati, & Sriman, 2006). Research activities on curcumin showed tremendous increase in the past few decades due to the discovery of its anticancer activity (Aggarwal, Kumar, & Bharti, 2003; Johnson & Mukhtar, 2007; Kunnumakkara, Anand, & Aggarwal, 2008; Kuttan, Bhunumathy, Nirmala, & George, 1985; Ruby, Kuttan, Dinesh Babu, Rajasekharan, & Kuttan, 1995; Wilken, Veena, Wang, & Srivatsan, 2011). Anticancer potential of curcumin originates from its ability to interact with several cellular targets NF-kb, protein kinase C and epidermal growth factor receptor tyrosine kinase, finally inducing apoptosis (Aggarwal & Shishodia, 2006; Sharma, Gescher, & Steward, 2005; Tomita et al., 2006).

Although, curcumin shows therapeutic efficacy towards a variety of diseases including cancer, its use as a drug is hampered owing to very low aqueous solubility and poor absorption. When it is absorbed, rapid metabolism and very fast elimination result in less bioavailability. Curcumin is extremely sensitive at physiological pH and encounters accelerated degradation (Wang et al., 1997). Several methods to address the shortcomings and to improve the therapeutic efficacy of curcumin have been explored. Strategies proven to be suitable for encapsulation and administration of curcumin includes polymeric nanoparticles (Bisht et al., 2007), polymer micelles (Iwunze, 2004) and vesicles (Sun et al., 2008).

The development of a polymer-drug conjugate provides a facile strategy to enhance the therapeutic efficacy of curcumin (Maeda, Seymour, & Miyamoto, 1992). Various biopolymers and water-soluble synthetic polymers have been exploited for conjugation of curcumin. Grafting of curcumin to a hydrophilic polymer is found to increase its solubility, permeability and stability, ultimately leading to the enhanced biological activity. Among the different types of hydrophilic polymers used for conjugation, polysaccharides have gained significant attention because of their biodegradability, biocompatibility, cost effectiveness and presence of large number of reactive functional groups for conjugation and further modification. Sreenivasan and coworkers reported the efficacy of polysaccharides such as alginate and hyaluronic acid (HA) for conjugation of curcumin, considering the hydrophilicity and biodegradability of alginate and HA’s affinity towards cell-specific surface markers such as CD44 (Dey & Sreenivasan, 2014; Manju & Sreenivasan, 2011). Both the polymer-curcumin conjugates formed...
micelle in aqueous medium and showed enhanced toxicity. PEG, a synthetic polymer is also widely used for conjugation of hydrophobic drugs (Yang et al., 2012).

In the present work, a novel polymer-drug conjugate is developed from gum arabic (GA) to augment the therapeutic efficacy of curumin towards cancer cells. This conjugate contains hydrophobic (curcumin) and hydrophilic (gum arabic) entities and hence self assembles to micelles with hydrophobic core and hydrophilic shell in aqueous medium. Gum arabic is a biocompatible, non-toxic, highly water soluble, natural gum obtained from acacia tree. It is a branched, complex polysaccharide containing arabinose, rhamnose, galactose and gluconic acid residues with backbone consisting of 1,3 linked β-D-galactopyranosyl units. The side chains are composed of two to five 1,3 linked β-D-galactopyranosyl units, joined to the main chain by 1,6 linkages (Verbeke, Dierckx, & Dewettinck, 2003). Asialoyglycoprotein receptor (ASGPR) on hepatocytes can specifically bind with ligands containing β-D-galactose and N-acetylgalactosamine residues (Wang et al., 2006; Wu, Nantz, & Zern, 2002; Zhou et al., 2012). In hepatocarcinoma cells, ASGPR is over expressed and it has been validated as a potential target for selective drug delivery to the liver cancer cells. Gum arabic contains galactose units in its complex structure, hence the malignant liver cells may exhibit enhanced binding and uptake of GA. Gum arabic-drug conjugates have been investigated for controlled drug delivery applications (Nishi, Antony, & Jayakrishnan, 2007a; Nishi et al., 2007b). Ayadi et al. (2010) developed gum arabic and chitosan based nanoparticle system for oral delivery of insulin.

In the present study, curcumin (Cur) is attached to gum arabic by a DCC/DMAp coupling reaction. Physicochemical properties of the gum arabic-curcumin conjugate (GA-Cur) are evaluated. To the best of our knowledge this is the first report on gum arabic-curcumin conjugate for delivery of curcumin to HepG2 cells exploiting the targeting efficiency of the galactose units on gum arabic.

2. Materials and methods

2.1. Materials

Gum arabic (from acacia tree), dimethylamino pyridine (DMAP) and N,N-dicyclohexyl carbodiimide (DCC), minimum essential medium (MEM), sodium bicarbonate and propidium iodide (PI) were obtained from Sigma–Aldrich, Saint Louis, USA. Foetal bovine serum (FBS) was procured from Invitrogen, USA. Sodium chloride, 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 Synthe Industries Ltd., Kolenchery, Kerala, India. Dialysis tubing (6000–8000, 3500 MWCO) was received from Spectrum Laboratories Inc., CA, USA.

2.2. Conjugation of curcumin to gum arabic

Curcumin was conjugated to gum arabic by a previously reported procedure (Manju & Sreenivasan, 2011) with slight modification. Briefly, 0.4 g (2.3 × 10⁻³ mol) of gum arabic was dispersed in anhydrous DMSO (50 mL) for 12 h by stirring magnetically. To this solution, 0.009 g of DCC (4.36 × 10⁻⁵ mol) and 0.005 g of DMAP (4.09 × 10⁻⁵ mol) were added and stirred for another 1 h at room temperature under nitrogen atmosphere to activate the carboxylic acid groups of GA. Curcumin (0.05 g, 1.35 × 10⁻⁴ mol) dissolved in DMSO (20 mL) was added to the above solution and was stirred for 6 h at 55–60 °C. After the reaction, the solution was dialyzed (MWCO 3500) for one day against DMSO and three days against distilled water to remove the unreacted curumin. Purified dialysate was freeze dried and kept under refrigeration. ¹H NMR spectra of GA-Cur and curcumin were recorded in DMSO-d₆ using 500 MHz spectrometer (Bruker Avance DRX 500).

2.3. Characterization of GA-Cur conjugate

2.3.1. Formation of GA-Cur conjugate micelle and its characterization

When dispersed in water, amphiphilic GA-Cur could self assemble into micelles. Size and zeta potential of these micelles were analyzed by dynamic light scattering (Malvern zetasizer, Nanoseries ZEN40002/SYS, UK). Conjugate (1 mg/mL) was dispersed in distilled water and measurements were performed at a scattering angle of 173°. Three measurements were recorded per sample with a time span of 11 s. For zeta potential determination, the same solution was used and three measurements were taken per sample. Size and morphology of GA-Cur micelle was analyzed by scanning electron microscopy (FEI Quanta FEG 200HR Scanning Electron Microscope) and transmission electron microscopy (FEI, Tecnai S twin microscope with accelerating voltage 300 kV). Samples for SEM were prepared by dissolving the conjugate in water (1 mg/mL). A drop of GA-Cur conjugate was placed on a glass slide and was allowed to air-dry at ambient temperature. The samples were sputter coated with gold and were observed under SEM. Sample preparation for TEM was carried out in a similar fashion as that for SEM with concentration of 1 mg/5 mL. The dispersion was placed on a copper grid and air dried for 2 days before imaging. The molecular mass analysis of gum arabic and GA-Cur conjugate was performed using Gel permeation chromatography (GPC) (Waters HPLC System) using 0.07 M disodium hydrogen phosphate as mobile phase with a flow rate of 1 mL/min. Dextran standards of molecular weight 9,000/25,000 Da and PEG 400 were used for relative calibration.

2.3.2. Estimation of curcumin conjugated to GA

A standard curve was generated using solutions of curcumin in DMSO-water mixture (1:1, v/v). UV–visible spectrophotometer (Carry100 UV–visible spectrophotometer, Melbourne, Australia) was employed for finding out the absorbance. A stock solution of curumin was prepared in DMSO-water mixture (1:1, v/v). From this stock solution (1 mg/1 mL), different concentrations ranging from 0.002 mg/mL to 1 mg/mL were prepared and their absorbance was measured at 430 nm. Absorbance of GA-Cur was measured and concentration of curumin in the conjugate was estimated from the standard curve.

2.3.3. Absorption and emission spectra of GA-Cur conjugate

The absorption spectra of GA-Cur and curumin solutions were recorded by visible spectrophotometer (Carry100 UV–visible spectrophotometer, Melbourne, Australia). The conjugate was dissolved in water with equivalent curumin concentration of 5 μg/mL. Fluorescence spectra of GA-Cur and pure curumin were recorded on an F-2500 fluorescence spectrophotometer (HITACHI, Tokyo). Both samples were dissolved in water with equivalent curumin concentration of 5 μg/mL of curumin. The excitation wavelength was 434 nm and the emission spectra were recorded from 450 nm to 700 nm with excitation and emission slit width of 5 nm.

2.3.4. Determination of critical micelle concentration

The critical micelle concentration (CMC) of the GA-Cur conjugate micelle was determined by fluorescence spectroscopy using pyrene as the fluorescence probe (Guo et al., 2013). Briefly, 5 μL of pyrene solution (6.0 × 10⁻⁵ M) in acetone was added to a series of vials proceeded by evaporation to remove acetone. Aqueous solutions of GA-Cur in concentrations ranging from 0.001 μg/mL to 0.5 μg/mL were added to the vials and sonicated in an ultrasonic bath for 40 min to equilibrate the pyrene and the conjugate,
and then left undisturbed overnight at room temperature. The final concentration of pyrene in each vial was 6.0 × 10⁻³ M. The slit widths for excitation and emission were set at 5 nm to obtain the emission spectra. The emission spectra (F=2500 fluorescence spectrophotometer, HITACHI, Tokyo) were recorded from 350 to 600 nm with an excitation wavelength of 334 nm. The intensity ratio (I₁₂₅₀/I₃₃₄) of pyrene fluorescence bands at 373 nm and 384 nm was plotted against the logarithm of conjugate concentration; the CMC was determined by taking the cross-point when extrapolating the intensity ratios.

2.3.5. Stability analysis and aqueous solubility of GA-Cur

Stability of the conjugate (1 mg/mL) and curcumin (1 mg/mL, dissolved by sonication) in physiological pH (7.4) and acidic pH (pH 4–6) was studied at varied time intervals by measuring the changes in the absorbance at 430 nm. The samples were dissolved in respective buffer solutions of pH 4–7.4 and maintained at 37°C. Aliquots were withdrawn from each solution at specified time interval (1 h) and the absorbance was recorded using UV–vis spectrophotometer.

2.3.6. In vitro drug release studies

Curcumin release from GA-Cur conjugate is performed at two different pH (7.4 and 5) for a period of 48 h (Anitha, Uthaman, Nair, Jayakumar, & Lakshman, 2013). A known amount of GA-Cur conjugate is dissolved in 3 mL of buffer of pH 5 and pH 7.4 and transferred to dialysis membrane. The membranes were immersed in the corresponding buffer (10 mL) release medium and incubated at 37°C. At predetermined time intervals, 3 mL of the release medium was withdrawn and replenished with fresh buffer. Released curcumin was dissolved in 1 mL of DMSO and quantified spectrophotometrically (Carry100 UV–visible spectrophotometer, Melbourne, Australia) from the standard curcumin curve.

2.3.7. Cytotoxicity studies

For evaluating the anticancer activity of GA-Cur conjugate, MTT assay was performed on MCF-7 and HepG2 cells. MCF-7 and HepG2 cells were procured from National Center for Cell Sciences, Pune. Cells were maintained in Eagles MEM supplemented with 2 mM glutamine, sodium bicarbonate (7.5%), 10% Foetal Bovine Serum (FBS) and 1% sodium pyruvate in an incubator set at 37°C, 5% CO₂ and >90% relative humidity. MCF-7 and HepG2 cells were seeded on a 96-well plate with a density of 10,000 cells/well and incubated at 37°C for 24 h. The test sample was prepared by dissolving GA-Cur in water and curcumin in DMSO. For in vitro assay, half dilutions of the stock GA-Cur (concentration of curcumin ranging from 25 to 0.75 μg/mL) and curcumin (concentration ranging from 25 to 0.75 μg/mL) were prepared in culture medium. Equal volumes (100 μL) of different dilutions of GA-Cur and curcumin were added to subconfluent cells and incubated for 24 h at 37°C. Cells supplied with normal culture medium was considered as cell control and curcumin was taken as assay control. Cells were rinsed with serum free MEM and incubated with 100 μL of MTT reagent (50 μg/mL in serum free medium) for 2 h. MTT reagent was removed and the formazan product formed was solubilized in 100 μL of isopropanol. The absorbance was measured using a multiwell plate reader (Biotek, USA) at 570 nm. Percentage cell activity was calculated and was analyzed statistically. The half maximal inhibitory concentration (IC₅₀) of GA-Cur conjugate in MCF-7 and HepG2 cells were calculated using Microsoft Excel.

2.3.8. Cellular uptake of the conjugate micelle

The uptake of GA-cur and free curcumin by HepG2 and MCF cells were determined by detecting the fluorescence after exposing the cells to a dose of 3.125 μg/mL for 24 h. Briefly, cells cultured on glass cover slips at a density of 1 × 10⁴ cells/cm² were exposed to GA-Cur and curcumin in serum free medium for 24 h. The cells were washed thrice with PBS and were fixed in 70% ethanol for minimum 24 h and the nucleus was counter stained with propidium iodide (10 μg/mL) for 1 min. The uptake was visualized under confocal laser scanning microscope (CLSM Meta 510Carl Zeiss, Germany). The cells were observed under confocal microscope under multi-channel mode using excitation wavelengths of 434 nm (blue diode for curcumin) and cells cultured in normal medium without particles were used to set the background.

2.3.9. Statistical analysis

All the experiments were performed in triplicate and the results are expressed as mean ± standard error (SD). Statistical analysis of the data was assessed by Student’s t-test. A value of *p < 0.05 was considered significant (n = 3).

3. Results and discussion

3.1. Preparation and characterization of GA-Cur conjugate micelle

Different types of nano carriers have been developed to overcome the limitations of curcumin. Nanogels, liposomes and nanoparticles are among the commonly used nano formulations (Gou et al., 2011; Ma et al., 2008; Mangalathillam et al., 2012; Yallapu, Gupta, Jaggi, & Chauhan, 2010). Polymer-drug conjugate strategies are also explored to improve the solubility and stability of curcumin. Solubility of curcumin so far achieved in aqueous medium by conjugation with a polymer is in the range of <852 μg/mL (Dey & Sreenivasan, 2014; Kim et al., 2011; Manju & Sreenivasan, 2011). Increasing the amount of conjugated curcumin would lead to enhanced therapeutic potency towards cancer cells.

In order to prepare the polymer-curcumin conjugate, a natural polysaccharide gum arabic is selected considering its high water solubility (can prepare solutions up to 55%, w/v), low cost, easy availability and the presence of galactose groups. Galactose is already identified as a targeting ligand for HepG2 cells since it will facilitate ASGPR mediated endocytosis in HepG2 cells. Scheme 1 summarizes the procedure for GA-Cur conjugate preparation. Hydroxyl group of curcumin is conjugated to the acid groups of GA by DCC/DMAP coupling reaction through an ester linkage.

Conjugation of curcumin (Fig. 1(A)) to GA is confirmed by ¹H NMR spectroscopy. ¹H NMR spectrum of the resultant conjugate (GA-Cur) (Fig. 1(B)) contains multiple proton resonance peaks of curcumin in the region between 6 and 8 ppm (Fig. 1(B), b–g) together with the distinctive singlet –OCH₃ proton peak at 3.84 ppm (Fig. 1(B), h). The spectrum also contains characteristic GA proton peaks (1.27 ppm and characteristic envelope around 3.99 ppm, Fig. 1(B), i) (Weinbreck, Rollema, Tromp, & de Kruijf, 2004).

Gum arabic has a number average molecular weight of 383,903 and after curcumin conjugation it is reduced to 367,953. Conjugation of curcumin molecules to gum arabic is expected to increase the molecular weight. Gum arabic is a branched polysaccharide and under the reaction conditions for curcumin conjugation, side chain cleavage might have occurred leading to the reduction in molecular weight.

Self assembly of biopolymers in aqueous medium depends upon its amphiphilicity (Akiyoshi & Sunamoto, 1996). The amphiphilic GA-Cur conjugate can be easily dispersed in aqueous medium which self assembles to micelles with hydrophobic curcumin core and hydrophilic GA shell. Self assembly of the conjugate is governed by minimization of the interfacial energy governed by the balance between the hydrophilic–hydrophobic interaction of gum arabic and curcumin (del Barrio et al., 2010).

The size of the micelle plays a vital role in the accumulation of conjugates in tumor cells. Tumor vasculature cut off deter-
mines the diffusion and accumulation of nanoparticles inside the tumor and it varies between tumors. Normally, the vascularature cut off is in the range of 200–800 nm. Therefore, polymer-drug conjugate with small size can easily enter into the tumor through enhanced permeability and retention (EPR) effect. For getting confirmation regarding the self assembly of GA-Cur conjugate and to know about the size of the micelle, particle size analysis is performed using dynamic light scattering (DLS). The size of the conjugate is found to be in the range of 270 ± 5 nm. The particle size in this range is ideal for promoting selective accumulation of GA-Cur micelle in cancer tissues by the EPR effect. Nano micelle formation of amphiphilic polymers like galactosylated O-carboxymethyl chitosan-graft-stearic acid conjugates, siRNA–PLGA hybrid conjugates and PEG–PCL–PEI triblock copolymers are reported in the

![Scheme 1. Conjugation of curcumin to gum arabic by DCC/DMAP coupling reaction.](image)

**Fig. 1.** $^1$H NMR spectrum of curcumin (A) and GA-Cur conjugate (B). GA-Cur shows peaks of curcumin in the region between 6.5 and 8 ppm together with the distinctive singlet $-\text{OCH}_3$ proton peak at 3.84 ppm.

![Fig. 2. SEM (A) and TEM (B) images of GA-Cur conjugate. Spherical morphology of the micelle is evidenced from the image.](image)
literature (Endres, Beck-Broichsitter, Samsonova, Renette, & Kissel, 2011; Guo et al., 2013; Lee, Mok, Lee, & Park, 2011). FE-SEM and TEM are used for the direct visualization of the morphology and size of the micelle. As illustrated in Fig. 2, FE-SEM (Fig. 2A) and TEM (Fig. 2B) images show the spherical morphology of the GA-Cur conjugate micelles. Size of the conjugate micelle obtained by SEM is 203 ± 10 nm. The variation in size observed between DLS and SEM measurements is due to the difference in the processing conditions. In SEM, GA-Cur is imaged in dry state, while in DLS, measurements are carried out in aqueous medium and the micelles are in swollen form resulting in higher hydrodynamic radius. Surface morphology of the conjugate micelle is well evident from TEM analysis. TEM image (Fig. 2B) represents spherical GA-Cur conjugate micelles with an average diameter of 200 ± 10 nm.

The zeta potential of GA-Cur is also measured by DLS after dispersing the conjugate in aqueous medium and shows a negative zeta potential of −36.4 mV. The negative charge is due to the carboxylic acid groups of GA and prevents GA-Cur micelles from aggregation. High negative zeta potential of the micelle indicates the stability of the nanoparticles in aqueous medium (Hans & Lowman, 2002). It has been reported in the literature that positively charged nanoparticles would be cleared rapidly from the circulation after intravenous and intraperitoneal administration (Malik et al., 2000) compared to the negatively charged particles. More than that, the negatively charged GA-Cur with hydrophilic GA shell may limit protein adsorption to a greater extent and enhance the circulation time with increased EPR effect leading to high antitumor efficacy.

Self-assembly behavior of GA-Cur conjugate is again investigated by measuring CMC of GA-Cur conjugate using pyrene as a hydrophobic fluorescent probe. Fluorescence characteristics of pyrene depend on the properties of the solubilizing medium and it exhibits different fluorescence behavior in micellar and nonmicellar solutions. Pyrene is hydrophobic and shows less fluorescence emission below CMC of micelle. Pyrene preferentially goes inside the hydrophobic core of polymer micelles; hence upon micelle formation it exhibits enhanced emission in aqueous solution. Emission spectra of pyrene encapsulated micelle are recorded and the intensity ratio of I_{337}/I_{374} of pyrene is evaluated as a function of GA-Cur conjugate concentration (Fig. 3). A rapid increase in the fluorescence intensity and a red shift in the absorption of pyrene are observed with increase in the concentration of GA-Cur. From the plot of intensity ratios (I_{337}/I_{374}), the CMC value is estimated to be 0.023 mg/mL.

Conjugation of curcumin to gum arabic is confirmed by measuring the UV absorption spectrum of the GA-Cur conjugate. Curcumin exhibits absorption in the UV–visible spectral region in distilled water with an absorption peak around 430 nm. GA-Cur shows absorption maximum at 434 nm corresponding to the presence of curcumin in the conjugate. Shift in the wavelength indicates the formation of conjugate. Ester linkage between GA and curcumin present in the GA-Cur conjugate is responsible for the red shift (Fig. 4A) (Manju & Sreenivasan, 2011).

The fluorescence spectrum of GA-Cur recorded in water is shown in Fig. 4B. It also proves the successful formation of the conjugate. Curcumin possesses intrinsic fluorescent properties. Shift in the emission (to higher wavelength, from 547 nm to 568 nm) for the conjugate compared to pure curcumin demonstrates the conjugation between the GA and curcumin (Manju & Sreenivasan, 2011).

The purpose of conjugation of curcumin to the hydrophilic polysaccharide or encapsulation of curcumin in micelles is to improve its aqueous solubility. Curcumin exhibits a solubility of 2.792 µg/mL (Kim et al., 2011) in aqueous medium which significantly limits its bioavailability. Kim et al. (2001) report that conjugation of curcumin to human serum albumin results in 300-fold increase in the solubility. Covalent conjugation of curcumin to hydrophilic polysaccharide such as hyaluronic acid and alginate step-up the solubility to 97.5 µg/mL and 109 µg/mL, respectively (Dey & Sreenivasan, 2014; Manju & Sreenivasan, 2011). In the present study, the amount of curcumin conjugated to gum arabic is determined from the standard curve plotted by dissolving curcumin in DMSO–water mixture and measuring the absorption intensity. It is found that 1 mg of the conjugate contains 13 µg of curcumin. Aqueous solubility of GA-Cur is determined and found that more than 200 mg of the GA-Cur can be solubilised in 1 mL of water which corresponds to 2.6 mg/mL of curcumin. Thus the conjugate tremendously increases the solubility of curcumin, since it is conjugated to a highly hydrophilic polysaccharide. To the best of our knowledge, this is the first report of a polymer–curcumin conjugate which shows 900-fold excess solubility than that of free curcumin. This immense solubility is attributed to the very high aqueous solubility of GA.

3.2. Stability studies of GA-Cur conjugate

One of the main disadvantages that restrict curcumin’s use as a drug is its stability in aqueous medium. At a pH above neutral, curcumin undergoes swift degradation first by hydrolysis, followed by molecular fragmentation within 30 min to various degradation products. A remarkable decrease in the absorption intensity of curcumin would be observed (Manju & Sreenivasan, 2011) as a result. The stability of GA-Cur in PBS buffer (Fig. 5A) at 37 °C is examined to analyze the influence of conjugation on the stability of the conjugated curcumin. Only a slight change in the absorbance is observed even after 5 h of incubation at 37 °C in PBS (pH 7.4), whereas curcumin degraded completely within 25 min (Fig. 5A). There is no significant difference in the absorbance of GA-Cur even after 5 h (*p = 0.65), whereas, when curcumin shows a significant decrease in the absorbance (*p = 0.003) after 5 h. In micelle, curcumin is present in the inner core and is protected from deprotonation and degradation in the physiological pH. The conjugation of curcumin to GA stabilizes curcumin against hydrolysis and increases its aqueous stability. The stability of the conjugate is also performed under acidic conditions since the conjugate is prepared for curcumin delivery to carcinoma cells (which has acidic environment). Stability of curcumin (Fig. 5B) is higher at acidic pH than that at neutral pH. GA-Cur conjugate (Fig. 5B) shows similar degradation kinetics at acidic pH also.
are after 3.3.

Curcumin and ester conjugate

later

7.4.

In studies

GA-Cur

Stability of curcumin

7.4

In Fig.

cells.

were dissolved in water with curcumin equivalent concentration of 5 μg/mL.

Fig. 5. Stability studies of GA-Cur and free curcumin in PBS at pH 7.4 (A) and under acidic conditions (B). After conjugation, stability of curcumin is enhanced. Data shown are mean value ± standard deviation (SD) (n = 3, *p < 0.05).

3.3. In vitro curcumin release from GA-Cur conjugate

Curcumin release from GA-Cur conjugate is analyzed at pH 5 and 7.4 and the release pattern is shown in Fig. 6. At acidic pH, the ester linkage between curcumin and GA breaks and higher curcumin release is observed at this pH. GA-Cur shows 60% release after 48 h at pH 5, while 40% curcumin release is observed at pH 7.4. The ester linkage between GA and curcumin is comparatively stable at neutral pH, therefore lesser release is observed. Hence GA-Cur conjugate is suitable for the delivery of curcumin to tumor cells, since these cells have acidic environment attributed to the accumulation of metabolic products in these cells due to poor blood vessel architecture.

3.4. Cytotoxicity studies

The anticancer activity of GA-Cur conjugate is evaluated in HepG2 and MCF-7 cells by MTT assay with free curcumin as assay control. The cells are exposed to the conjugate in six doses (25–0.78 μg/mL) for 24 h and cell activity is evaluated (Fig. 7). The results confirm that the doses 25–3.125 μg/mL show severe cytotoxicity (<20% metabolic activity) to HepG2 cells. However, the concentration 3.125 μg/mL is noncytotoxic to MCF-7, but cytotoxic to HepG2 cells. IC50 values of GA-Cur in HepG2 and MCF-7 cells are 2.29 and 4.07 μg/mL, respectively. The cytotoxic potential of GA-Cur is evident while comparing the cellular activity with the assay control (free curcumin). Curcumin shows IC50 values of 3.8 μg/mL and 5.6 μg/mL in MCF-7 and HepG2 cells, respectively. Higher toxicity of GA-Cur to HepG2 cells is due to the presence of galactose moiety in the structure of gum arabic which can selectively identify asialoglycoprotein receptor (ASGPR) on the surface of hepatocytes (Rigopoulos et al., 2012). The results demonstrate that the conjugate shows effective anticancer activity towards MCF-7 and HepG2 cells. The cytotoxicity of GA-Cur is due to the enhanced water solubility and cell internalization ability. Effective exposure time (EET) of GA-Cur conjugate is augmented as a result of enhanced solubility, where as curcumin experiences very short EET.

3.5. In vitro cellular uptake studies of GA-Cur conjugate

The in vitro cellular uptake of GA-Cur conjugate by HepG2 and MCF-7 cells for 24 h is analyzed by confocal laser scanning microscopy (CLSM) (Fig. 8). GA-Cur shows cellular uptake in both the cancer cell lines. GA-Cur is internalized in the cytoplasm and nucleus of HepG2 (Fig. 8C) and MCF-7 cells (Fig. 8D). Free curcumin is located both in the cytoplasm and nucleus of the HepG2 cells.
Concentration MCF-7 sis conjugate

Fig. (High 2012 to (enhanced fluorescence cell cytotoxicity A

A)

MTT assay. GA-Cur shows selective toxicity towards HepG2 cells. Data shown are mean value ± SD (n = 3, *p < 0.05).

Fig. 7. Cytotoxicity of GA-Cur at varying concentrations of 25–0.78 μg/ml. HepG2 and MCF-7 cells were incubated with GA-Cur for 24 h and the cell activity was determined by MTT assay. GA-Cur shows selective toxicity towards HepG2 cells. Data shown are mean value ± SD (n = 3, *p < 0.05).

Fig. 8. Cell uptake studies of GA-Cur and free curcumin in HepG2 cells (A and C) and MCF-7 cell lines (B and D) for 24 h. Nucleus of the cells were counter stained with PI. Concentration of GA-Cur and free curcumin used for these studies is 3.125 μg/ml.

(Fig. 8A) and MCF-7 cells (Fig. 8B) (Saab et al., 2011; Yang et al., 2012). Low fluorescence intensity of free curcumin in both the cells (Fig. 8A and B) is attributed to its poor uptake and high metabolism. High fluorescence of GA-Cur in HepG2 cells may be explained due to ASGPR mediated increased cellular uptake. The CLSM analysis reveals that the conjugate shows higher fluorescence intensity compared to free curcumin, which is assumed to be the result of enhanced solubility and efficient cell internalization ability. These results indicate that internalization of curcumin is increased after conjugation with GA. Higher solubility and facile passage of the conjugate through the cell membrane provide the cells efficient drug internalization capability with a longer EET. Better internalization would be favorable to the enhanced cytotoxicity of GA-Cur conjugate.

4. Conclusions

A novel GA-Cur conjugate has been successfully developed to address the limitations of curcumin such as poor solubility and stability. The conjugate shows 900-fold higher solubility than that of free curcumin. GA-Cur self assembles to micelle in aqueous medium due to the hydrophilic and hydrophobic interaction between GA and curcumin. Being occupied in the inner core of the micelle, curcumin is protected from the external physiological acquaintance, in which it degrades quickly. Hence the micelle structure enhances the stability of curcumin in aqueous medium. MTT assay reveals the higher cytotoxicity of GA-Cur towards HepG2 cells than that of MCF-7 cells, thereby confirming the targeting efficacy of galactose on GA. The cellular uptake study shows that GA-Cur conjugate can successfully transport the drug to the cytoplasm of HepG2 and MCF-7 cells. This new conjugate has the capability of overcoming the obstacles associated with curcumin and is a promising drug delivery system.

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