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Synthesis, characterization, and in vitro evaluation of palmitoylated arabinogalactan with potential for liver targeting

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ABSTRACT

Arabinogalactan (AG), a water soluble polysaccharide with more than 80 mol % galactose units, was hydrophobized by covalent attachment of palmitoyl chains using a base-catalyzed esterification reaction with the objective of effective amalgamation of arabinogalactan in liposomes for targeting asialoglycoprotein receptors (ASGPR) on liver parenchymal cells. Palmitoylated AG (PAG) was characterized by physico-chemical parameters, IR, ^{1}H NMR, and ^{13}C NMR and molecular weight determination by gel permeation chromatography. PAG was incorporated in liposomes and the liposomes were characterized by dynamic light scattering, optical microscopy, zeta potential, and transmission electron microscopic (TEM) techniques. The liposomal system was evaluated for acute toxicity in swiss albino mice and was found to be safe. Targeting ability of PAG was confirmed by in vitro binding affinity to *Ricinus communis* agglutinin (RCA_{120}), a lectin specific for galactose. The liposomal system with PAG was evaluated for cytotoxicity on HepG2, MCF7, and A549 cancer cell lines. Cytotoxicity study revealed enhanced activity on ASGPR-expressive HepG2 cells as compared to MCF7.

1. Introduction

Delivering drugs or bio-active molecules in various macromolecular carriers like liposomes, nanoparticles etc. have gained substantial importance due to the targeting potential of these carriers. Liposomes are the widely used macromolecular carriers for delivery of lipophilic drugs[1] as well as hydrophilic drugs.[2] Upon intravenous administration, conventional liposomes are cleared by the reticuloendothelial system limiting its use to passive targeting based on its size[3] and lipid composition.[4] Various ligands that have been incorporated in liposomes and investigated include anionic and cationic agents for charge interaction,[5] monoclonal antibodies to specific antigens,[6] and various carbohydrates like pullulan,[7] dextran,[8] amylopectin,[9] and scleroglucan[10] for actively targeting carbohydrate receptors on various cell types. Carbohydrates, owing to their natural origin, have better ability to bind to receptors on various cell types and can also provide a stealth protection[11] to the macromolecular carrier like liposome.

Hepatic targeting is a challenging area to the formulation scientist. Liver is the major site of accumulation for macromolecular/particulate carriers;[12] yet these carriers lack the desired therapeutic effect in diseases/disorders of the liver like hepatocellular carcinoma, liver cirrhosis, hepatitis, hepatic tuberculosis etc. The macromolecular/particulate carriers accumulate in the non-parenchymal cells (Kupffer cells) of the liver[13] whereas the disorders/diseases of the liver are confined to the parenchymal cell. Parenchymal cells have carbohydrate sensitive receptors called ASGPR that recognize *N*-acetylgalactosamine and β-D-galactose units[14,15] as the major signaling unit.[16] Specificity and selectivity of monosaccharides and disaccharides containing galactose as a ligand targeting ASGPR are reported.[17,18] Larch arabinogalactan obtained from *Larix occidentalis* (AG) is a polysaccharide with high content ~80 mol % of galactose unit.[19] AG has been reported for its anti-metastatic activity by stimulation of production of natural killer cells.[20] Amalgamation of AG with high galactose content on the surface of liposomes could lead to a better receptor ligand interaction and in conjunction with an anti-cancer agent, can help in better management of hepatocellular carcinoma wherein an over-expression of ASGPR is reported.[21]

This work was aimed at the synthesis and characterization of a palmitoyl derivative of AG with potential to target the ASGPR on the parenchymal cells of the liver. PAG was characterized by advanced analytical techniques and incorporated into liposomes which were characterized by dynamic light scattering, optical microscopy and TEM. Cytotoxicity of PAG liposomes was studied on HepG2, MCF7 and A549 cancer cell lines. Receptor binding affinity of PAG liposomes was assessed by its binding with *Ricinus communis* agglutinin (RCA_{120}).

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2. Results and discussion

2.1. Synthesis of PAG

AG is a US FDA approved dietary fiber. AG with its high galactose content has liver specificity,[22–24] but its potential as a liver directing ligand for macromolecular carriers like liposomes has not been reported. In this study AG was hydrophobized by reacting palmitoyl chloride with AG in a base catalyzed esterification reaction. Physical properties of AG and PAG are shown in Table 1. The % yield of PAG was found out to be 40% with respect to the total weight of palmitoyl chloride and AG used in reaction. There was a change in the solubility pattern for AG (soluble in water and DMSO) and PAG (soluble in chloroform and diethyl ether) confirming palmitoylation. The mp of PAG was 62.5 °C. AG has been reported with a diverse range of molecular weights, from 7.5 to 37 kDa,[25,26] based on the method of extraction employed and the method of analysis used for the determination of molecular weight. The weight average molecular weight of PAG by Gel Permeation Chromatography (GPC) was found to be 24,453 Da.

2.2. Characterization of PAG

2.2.1. Fourier-transform infrared spectroscopy (FTIR)

FTIR spectra of the two reactants AG and palmitoyl chloride along with the spectrum of PAG are shown in Fig. 1. AG showed a prominent band at 3400 cm^{-1} characteristic of hydroxyl group of monosaccharide units of AG. Palmitoyl chloride showed a prominent band at 2800 cm^{-1} characteristic of $-CH_2-$ stretch of its alkyl chain. Two prominent bands in FTIR spectrum of PAG at 1747.66 and 1165.11 cm^{-1} are characteristic of an ester group. Two bands at 2853 and 1468 cm^{-1} in the spectrum of PAG represent the $-CH_2-$ stretching and bending vibrations of the palmitoyl chain, respectively. The intensity of band characteristic for a hydroxyl group at ~3400 cm^{-1} in PAG reduced in comparison to AG indicating the formation of ester bond between the hydroxyl group of monosaccharide unit of AG and carboxyl group of palmitoyl chloride. The shift of C=O of palmitoyl chloride from 1801 to 1747 cm^{-1} was an indication of change in functionality from an acid chloride to an ester bond.

Table 1
Physical properties of AG and PAG

Physical characteristic	AG	PAG
Color	White solid	Buff solid
Odor	Slight woody	Odorless
Solubility	Soluble in water, DMSO. Insoluble in chloroform, diethyl ether	Soluble in chloroform and diethyl ether. Insoluble in water and DMSO
mp	Degrades above 200 °C	62.5 ± 2.5 °C

2.2.2. ^{1}H NMR

The ^{1}H NMR spectra of AG (blue) and PAG (red) are shown in Fig. 2A. The chemical shift of hydrogen atoms of the palmitoyl chain is observed in the spectrum of PAG viz. peak at δ_H 0.89 in Fig. 2A is of terminal $-CH_3$ protons (carbon C_1) of the palmitoyl chain and peaks from δ_H 1.26 to 2.34 are of 14 $-CH_2-$ protons (carbon C_2–C_{15}) of the palmitoyl chain. The chemical shift of the protons representing the carbohydrate backbone in AG and PAG was observed between δ_H 3.00 and 5.39.

2.2.3. ^{13}C NMR

The ^{13}C NMR spectra of AG (blue) and PAG (red) are shown in Fig. 2B. The chemical shift of carbon atoms present in the palmitoyl chain is observed in the spectrum of PAG viz. δ_C 160.1 for the carboxyl group (carbon C_{16}) of ester bond between palmitoyl chloride and hydroxyl group of AG, δ_C 14.1 for the terminal $-CH_3$ carbon (carbon C_1) atom of methyl group in palmitoyl chain, and δ_C 22.6–33.9 for the 14 methylene carbon atoms (carbon C_2–C_{15}) of the palmitoyl chain. The chemical shift of carbon atoms of the carbohydrate backbone was observed in the region between δ_C 48.0 and 110.0. NMR confirmed the intact nature of the carbohydrate backbone of the AG, evident from similar NMR spectra in the region of carbohydrate (δ_H 3.0–5.4 in ^{1}H NMR and δ_C 48.0–110.0 for ^{13}C NMR).

2.3. Characterization of liposomes

PAG was incorporated into the liposomes by a reverse phase evaporation method. Liposomes with and without PAG were

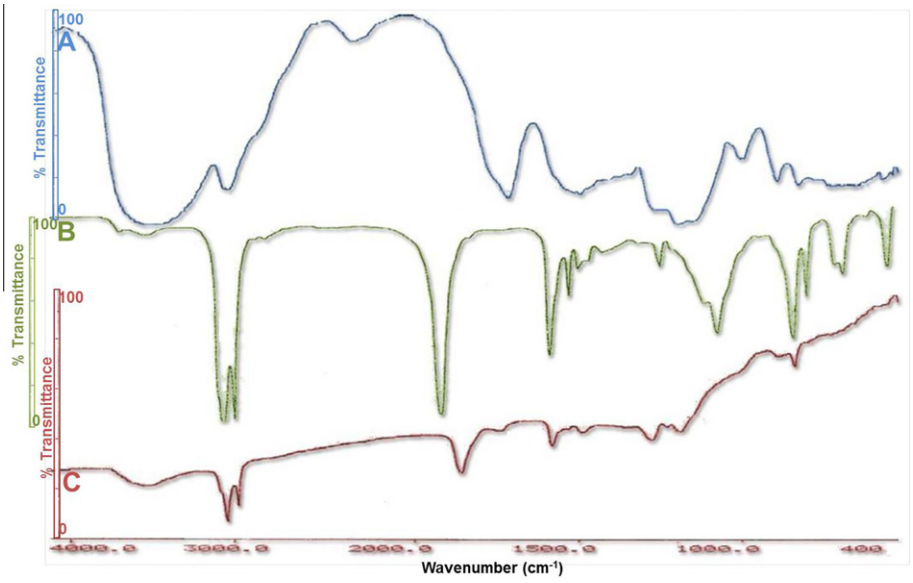

Figure 1. FTIR spectra of AG (A, blue), palmitoyl chloride (B, green) and PAG (C, brown).