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RESEARCH ARTICLE
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SYNTHESIS OF PALMITOYL CHLORIDE MANNOSE CONJUGATE AS TARGETING LIGAND FOR LIVER TARGETING OF CAPACITABINE LOADED LIPOSOMES

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Abstract
Nanotechnology mediated drug delivery is utilize nowadays for effective drug delivery at desired cell target. Many scientific investigators have utilized nanocarrier mediated drug delivery of antitumor drug for active targeting of encapsulated drug at desired target cells. Capacitabine is approved anticancer drug especially use for management of breast and colon cancer. Various conventional drug delivery systems of capacitabine are available for clinical use. However, limited oral bioavailability is major hurdle associated with conventional delivery of capacitabine. Thus, present study has started with aim to synthesize palmitoyl chloride mannose conjugate for hepatocyte targeting of capacitabine loaded liposomes.

Keywords: Capacitabine, Liposomes, Palmitoyl chloride mannose conjugate, hepatocyte targeting

Introduction

Cancer is uncontrolled cell proliferation that aggressively invades other parts of the body. According to the International Agency for Research on Cancer (IARC), 14.1 million new cases of cancer were estimated to occur in 2012, with almost 8.2 million mortality cases. Based on IARC estimates, cancer is the 2nd most common cause of death in both economically developing and developed countries. About 13% of global cancer cases are estimated to have occurred in southwestern of Asia. The main cancer treatment modality, chemotherapy, has limitations that including various side effects (Nazari-Vanani et al., 2019).

The nanotechnology mediated drug delivery is widely use nowadays. Various nanocarriers like liposomes (Sansare et al., 2021), niosomes, ethosomes, transfersomes, glycerosomes, nanoparticles (Gupta et al., 2021), solid lipid nanoparticles (Sansare et al., 2020), nanostructured lipid carriers (Gupta et al., 2022), microspheres (Abrar, 2020) etc. are utilized for effective drug delivery. The drug loaded in nanocarriers is release in controlled manner. In addition to this, the cell specific targeting of loaded drug is one more advantage of nanocarrier based drug delivery system.

Capecitabine is used nowadays in the management of colon and breast cancer. It is used alone or in combination with other medicine (Nazari-Vanani et al., 2019). Inside the body, capecitabine gets converted into 5-fluorouracil. 5-fluorouracil hampers the formation of RNA and DNA in the cancer cells which eventually minimize the growth of cells. The major drawback of capecitabine is limited oral bioavailability.

These colloidal carriers offer various advantages including easy surface conjugation with targeting ligands for cell specific delivery. Hepatocyte specific drug delivery is a challenging concept to the formulation scientists (Pranatharthiharan et al., 2017). The carbohydrate receptors expressed on hepatic cells are called as asialoglycoprotein receptor. These receptors specifically recognize carbohydrates. Thus, present study has started with aim to synthesize palmitoyl chloride mannose conjugate for hepatocyte targeting of capacitabine loaded liposomes.

Materials and methods

Capecitabine was purchased from Naprod Life Sciences Pvt. Ltd (India). Palmitoyl chloride, dimethyl formamide and mannose were purchased from S. D. Fine Chemicals Ltd. (India). Dialysis membrane was purchased from Himedia (India). All other regents, chemicals and solvent were laboratory grade and purchased locally.

Synthesis and characterization of targeting ligand

The mannose was anchored to palmitoyl chloride to form hepatocytes targeting ligand. The hepatocytes of liver overexpressed carbohydrate receptors in case of liver cancer. Thus, mannose palmitoyl chloride conjugate as used to target drug loaded liposomes to hepatocyte.

The conjugate was synthesized according to previously published method (Shah et al., 2013). Palmoyled mannose was synthesized by esterification reaction. The reaction between hydroxyl group of the mannose and long fatty acid chain of palmitoyl chloride was used to form conjugate between palmitoyl chloride and mannose. The pyridine was used as catalyst in present reaction. Mannose (0.216 g) was dissolved in 5 ml of dimethyl formamide and stirred in round bottom flask at 80^0C for 1 hour by attaching nitrogen gas balloon to maintain oxygen free environment. The 1 ml of pyridine was added in resulting solution. The palmitoyl chloride (1.61 g) was dissolved in 2 ml of dimethyl formamide and resulting solution was added in mannose solution and stirred in round bottom flask. The resulting solution was stirred at 90^0C for 5 hours for completion of reaction. The solid product was separated by filtration and washed with 70% ethanol. The synthesized ligand was characterized with respect to infrared spectroscopy and proton NMR.

Fourier-transform infrared spectroscopy (FTIR)

Three FTIR spectrums of mannose, palmitoyl chloride and synthesized targeting ligand were recorded separately using FTIR spectrometer (Jasco FTIR-5300, Japan). Briefly 5 mg of samples were mixed with 300 mg potassium bromide separately and compressed into a thin disk in a die at pressure of 10 ton to form thin KBr plate. The resulting KBr disc was placed in spectrometer to record spectrum. The FIIR spectrum of two reactants i.e. mannose and palmitoyl chloride were also recorded to compare spectroscopic peaks of reactants with peaks of synthesized targeting ligand.

Proton nuclear magnetic resonance (^{1}H NMR)

^{1}H NMR spectrum of synthesized synthesized targeting ligand was recorded using NMR spectrometer (Bruker Avance II 400 NMR spectrometer) at frequency 400 MHz. The chloroform was used as solvent for dissolution of synthesized targeting ligand before recording ^{1}H NMR spectrum.

Formulation of capacitabine loaded conventional liposomes

Conventional liposomes were formulated by reverse phase evaporation technique (González-ortega et al., 2020). Lipoid S-90 and cholesterol were used as lipid phase for formation of drug loaded liposomes. Phosphate buffer (pH 7.2) was used as aqueous phase. Briefly, Lipoid S-90 and Cholesterol (75:25 % ratio) were dissolved in chloroform. The resulting lipid solution was added in aqueous phosphate buffer to form water in oil emulsion. The resulting emulsion was sonicated using probe sonicator (Sonic Vibra Cells). The chloroform from resulting emulsion was removed by rotary evaporator (Buchi Rotavapor R-114) under reduce pressure the temperature of water bath is

maintained at 40^0C and the rotation speed of flask was adjusted to 100 rpm to form a thick lipid gel. The formed gel was then redispersed in phosphate buffer (pH 7.2) to form liposomal vesicles. The resulting liposomal vesicles then sonicated using probe sonicator for further size reduction. The liposomal dispersion was filled in glass vial, kept in ice bath and subjected to sonication to prevent heating due to sonication.

Formulation of capacitabine loaded mannose conjugated liposomes

The mannose liposomes were formulated by reverse phase evaporation technique (Marwah et al., 2020). Lipoid S-90 and cholesterol were used as lipid phase for formation of drug loaded liposomes. Phosphate buffer (pH 7.2) was used as aqueous phase. Briefly, Lipoid S-90, Cholesterol and palmitoyl chloride mannose conjugate (75:15:10 % ratio) were dissolved in chloroform. The resulting lipid solution was added in aqueous phosphate buffer to form water in oil emulsion. The resulting emulsion was sonicated using probe sonicator (Sonic Vibra Cells). The chloroform from resulting emulsion was removed by rotary evaporator (Buchi Rotavapor R-114) under reduce pressure the temperature of water bath is maintained at 40^0C and the rotation speed of flask was adjusted to 100 rpm to form a thick lipid gel. The formed gel was then redispersed in phosphate buffer (pH 7.2) to form liposomal vesicles. The resulting liposomal vesicles then sonicated using probe sonicator for further size reduction. The liposomal dispersion was filled in glass vial, kept in ice bath and subjected to sonication to prevent heating due to sonication.

Results and Discussion

Synthesis and characterization of targeting ligand

The mannose was anchored to palmitoyl chloride to form hepatocytes targeting ligand. The hepatocytes of liver overexpressed carbohydrate receptors in case of liver cancer. Thus, mannose palmitoyl chloride conjugate as used to target drug loaded liposomes to hepatocytes. The conjugate was synthesized according to previously published method. Palmoyled mannose was synthesized by esterification reaction. The synthesized ligand was characterized with respect to infrared spectroscopy and proton NMR.

Fourier-transform infrared spectroscopy (FTIR)

Three FTIR spectrums of mannose, palmitoyl chloride and synthesized targeting ligand were recorded separately using FTIR spectrometer (Jasco FTIR-5300, Japan). Figure 1 represents FTIR spectrum of mannose. In spectrum of D-Mannose broad peak at 3398 cm^{-1} and intense peak at 2926 cm^{-1} indicate the presence of –OH stretching and $–CH_2$ stretching vibrations. Vibrational signals at 1064 and 1638 cm^{-1} indicate C=O stretching of either alcohol or aldehyde groups in mannose.

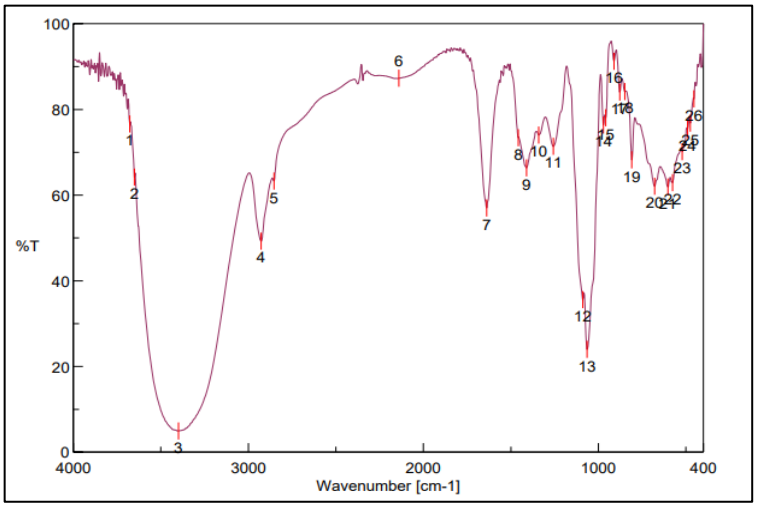

Figure 1 FTIR spectrum of mannose

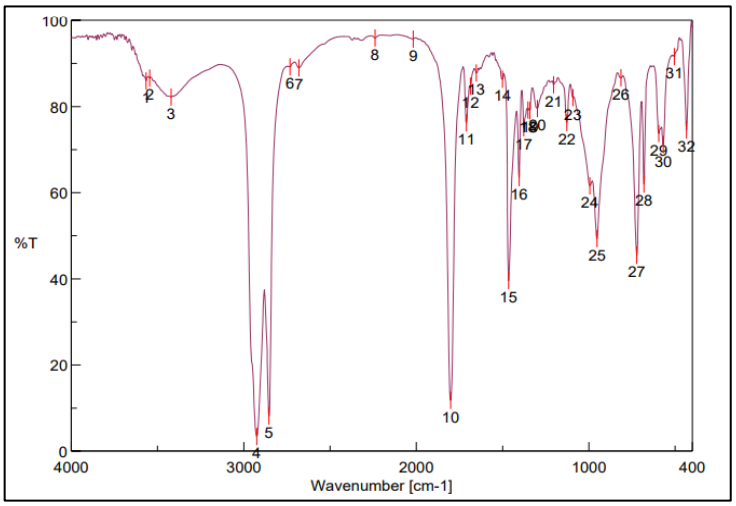

Figure 2 FTIR spectrum of palmitoyl chloride

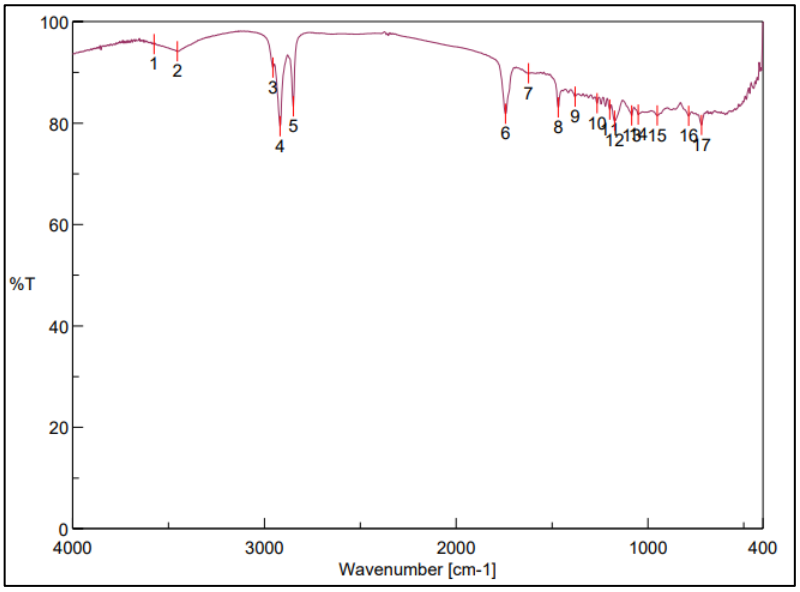

Figure 3 FTIR spectrum of synthesized ligand

Figure 2 represents FTIR spectrum of palmitoyl chloride. The spectrum of palmitoyl chloride revealed two sharp peaks at 2924.52 and 2854.13 cm^{-1} which indicates stretching of long chain CH_2 groups. The sharp peak at 1801.19 cm^{-1} indicates stretching of C=O group of acid chloride. The one sharper peak at 1465.63 cm^{-1} indicates CH_2 bending of long chain hydrocarbon. Figure 3 represents FTIR spectrum of synthesized ligand. The spectrum showed peaks of both mannose as well as palmitoyl chloride. In addition to this, the spectrum revealed one peak at 1742.37 cm^{-1} which indicated peak of ester functional group. Thus, appearance of peak of ester functional group clearly showed formation of ester bond between mannose and palmitoyl chloride.

Proton nuclear magnetic resonance (^{1}H NMR)

^{1}H NMR spectrum of synthesized synthesized targeting ligand was recorded using NMR spectrometer (Bruker Avance II 400 NMR spectrometer) at frequency 400 MHz. The chloroform was used as solvent for dissolution of synthesized targeting ligand before recording ^{1}H NMR spectrum. The NMR spectrum of synthesized ligand is represented in figure 4. The NMR spectrum revealed signal at 2.2 ppm which indicates ester linkage between mannose and palmitoyl chloride.

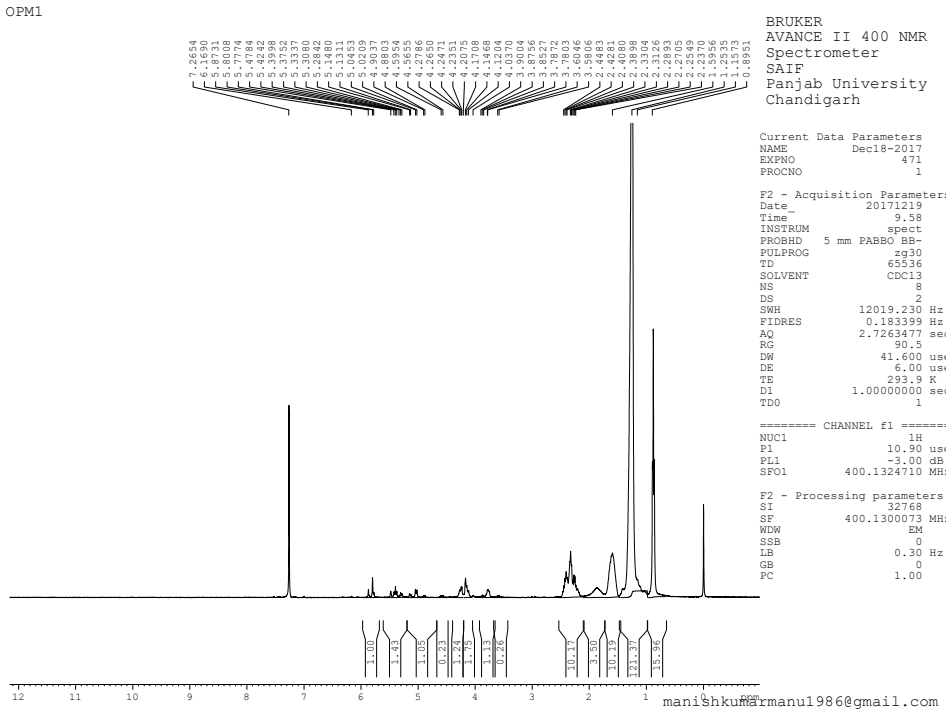

Figure 4 Proton NMR spectrum of synthesized ligand.

Formulation of capacitabine loaded liposomes

The reverse phase evaporation technique was utilized for fabrication of capacitabine loaded liposomes. Lipoid S-90 and cholesterol were used as lipid phase for formation of drug loaded liposomes. Phosphate buffer (pH 7.2) was used as aqueous phase. The mannose conjugation on surface of capacitabine loaded liposomes was done by addition of palmitoyl chloride mannose conjugate in lipid phase while preparation of liposomes.

Conclusion

The aim for present study was to synthesize palmitoyl chloride mannose conjugate as targeting ligand for liver targeting of capacitabine loaded liposomes. The palmitoyl chloride mannose conjugate was synthesize by reaction between palmitoyl chloride and mannose. The FTIR and proton NMR were used to confirm correctness of synthesis procedure adopted. The formulated targeting ligand was incorporated in capacitabine loaded liposomes. However, additional studies are required to confirm the targeting ability of synthetic ligand.

Reference

1. Abrar, A., 2020. Formulation and evaluation of microsphere of antiulcer drug using Acacia nilotica gum. Int. J. Health Sci. (Qassim). 14, 10–17.
2. González-ortega, R., Luka, Š., Skrt, M., Daniela, C., Mattia, D., Pittia, P., 2020. Liposomal Encapsulation of Oleuropein and an Olive Leaf Extract : Molecular Interactions , Antioxidant Effects and Applications in Model Food Systems. Food Biophys. https://doi.org/https://doi.org/10.1007/s11483-020-09650-y
3. Gupta, M.K., Sansare, V., Shrivastava, B., Jadhav, S., 2021. Design , fabrication and characterization of sesamol loaded polymeric nanoparticles : In vivo hepatoprotective potential in Wistar rats. Nanomedicine Res. J. 6, 296–303. https://doi.org/10.22034/nmrj.2021.03.010
4. Gupta, M.K., Sansare, V., Shrivastava, B., Jadhav, S., Gurav, P., 2022. Fabrication and evaluation of mannose decorated curcumin loaded nanostructured lipid carriers for hepatocyte targeting: In vivo hepatoprotective activity in Wistar rats. Curr. Res. Pharmacol. Drug Discov. https://doi.org/https://doi.org/10.1016/j.crphar.2022.100083

5. Marwah, M., Narain Srivastava, P., Mishra, S., Nagarsenker, M., 2020. Functionally engineered 'hepato-liposomes': Combating liver-stage malaria in a single prophylactic dose. Int. J. Pharm. 587, 119710. https://doi.org/10.1016/j.ijpharm.2020.119710
6. Nazari-Vanani, R., Karimian, K., Azarpira, N., Heli, H., 2019. Capecitabine-loaded nanoniosomes and evaluation of anticancer efficacy. Artif. Cells, Nanomedicine Biotechnol. 47, 420–426. https://doi.org/10.1080/21691401.2018.1559179
7. Pranatharthiharan, S., Patel, M.D., Malshe, V.C., Gorakshakar, A., Madkaikar, M., Ghosh, K., Padma, V., Pranatharthiharan, S., Patel, M.D., Malshe, V.C., Pujari, V., Gorakshakar, A., 2017. Asialoglycoprotein receptor targeted delivery of doxorubicin nanoparticles for hepatocellular carcinoma. Drug Deliv. 24, 20–29. https://doi.org/10.1080/10717544.2016.1225856
8. Sansare, V., Gupta, M.K., Shrivastava, B., Jadhav, S., Gurav, P., 2021. Comprehensive review on use of phospholipid based vesicles for phytoactive delivery. J. Liposome Res. https://doi.org/10.1080/08982104.2021.1968430
9. Sansare, V., Warrier, D., Shinde, U., 2020. Cellular trafficking of nanocarriers in alveolar macrophages for effective management of pulmonary tuberculosis. J. Tuberc. 3, 1016.
10. Shah, S.M., Pathak, P.O., Jain, A.S., Barhate, C.R., Nagarsenker, M.S., 2013. Synthesis , characterization , and in vitro evaluation of palmitoylated arabinogalactan with potential for liver targeting. Carbohydr. Res. 367, 41–47. https://doi.org/10.1016/j.carres.2012.11.025