Development of Liposome Formulation for the Co-delivery of Irinotecan and Safingol

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ABSTRACT

Liposomal formulations for the co-delivery of Irinotecan (CPT-11) and Safingol (L-threo-dihydrosphingosine) were developed and analysed to determine if these liposomes exhibited comparable drug uptake efficiencies, drug release and drug stability levels to liposomes produced without Safingol (Saf). 1,2-distearoyl-snglycero-phosphocholine/Cholesterol (DSPC/Chol) liposomes were formulated in the absence and presence of Saf and loaded with Irinotecan at two different drug-to-lipid mole ratios (D/L) - 0.1 & 0.025. Three batches of each liposomal formulation were made to obtain the relevant data. Results showed that DSPC/Chol liposomes formulated with Saf had comparable drug encapsulation rates as well as drug stability levels to those without. In addition, liposomes with Saf displayed greater drug release rate and extent than those without. Successful co-delivery of Irinotecan and Saf was thus developed.

INTRODUCTION

Irinotecan (CPT-11) is a water-soluble camptothecin derivative that exhibits anti-cancer activity. Camptothecins stabilize the covalent complex formed between Topoisomerase I and DNA which ultimately leads to apoptosis. As the active lactone form of irinotecan undergoes rapid hydrolysis at physiological pH to form an inactive carboxyl form, liposomes encapsulating irinotecan in its aqueous core have been formulated. By maintaining the aqueous environment at low pH, the lactone form of the drug can thus be preserved longer. The use of protein kinase C (PKC) inhibitors in combination with chemotherapeutic agents has been researched. One PKC inhibitor is Safingol (L-threo-dihydrosphingosine) (Saf), which has been shown to enhance the cytotoxic effect of anti-cancer drugs.

The goal of this study is to investigate if Irinotecan and Saf can be successfully co-encapsulated into liposomes. Firstly, the loading efficiency of Irinotecan into 1,2-distearoyl-snglycero-phosphocholine/Cholesterol (DSPC/Chol) liposomes with or without Saf is compared. To provide conditions for optimum drug loading, liposomes were first prepared in CuSO₄ solutions and later exchanging the external buffer to SHE buffer pH 7.5 (300mM sucrose, 20mM HEPES, 15mM EDTA). The divalent cation ionophore A23187 is then used to create the pH gradient via exchanging Cu²⁺ with protons to allow irinotecan uptake into the liposomes. The release profile for irinotecan from the liposomes will be determined by incubating the samples at 37°C and adding an equal volume of Fetal Bovine Serum (FBS) to each sample. Last of all, stability studies of the liposomes will be monitored for 4 weeks after drug loading.

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MATERIALS AND METHODS

Materials
Irinotecan was purchased from National University of Singapore Hospital Pharmacy. 1,2-distearyl-sn-glycero-phosphocholine (DSPC), Cholesterol and Safingol (L-threo-dihydrosphingosine) was purchased from Avanti Polar Lipids. Fetal bovine serum was from Hyclone. All other reagents were from Sigma-Aldrich. All reagents used are of analytical grade.

Liposome Preparation
DSPC/Chol (60:40 mol%) or DSPC/Chol/Saf (50:40:10 mol%) liposomes were prepared as follows. The lipids were weighed out (total mass = 50.0mg), dissolved in chloroform and dried to a thin film under low-pressured N₂ gas before being placed in high vacuum for ≥3 h. Lipid films were then hydrated (50.0mg/ml) with CuSO₄·5H₂O buffer (300mM, pH 4.05) and incubated (65°C, 1 h). Suspensions were extruded through stacked polycarbonate filters (0.08 and 0.1μm pore size) at 65°C for 10 times. Extruded sample used Sephadex G-50 size exclusion chromatography to exchange the external buffer with SHE buffer pH 7.5.

Loading of Irinotecan into preformed Liposomes
The ionophore A23187 (0.2 μg/μmol of lipid) was pre-incubated (60°C; 10 min) with the liposome sample. Irinotecan was added into the liposome samples at drug-to-lipid ratios (D/L) of 0.1 and 0.025. At various time points, 100μl aliquots of each sample were taken and spun down 1ml Sephadex G-50 spin columns (equilibrated with SHE buffer pH 7.5) at 680g for 3 min. The fractions were collected for lipid and irinotecan concentration determination.

Phosphate Assay: Lipid Concentration Determination.
The eluted samples (50μl) were mixed with 700μl Perchloric acid. The mixtures were heated at 190°C for 1 h. Following, samples were cooled and 700μl Friske Solution (1.44M NaHSO₃, 0.04M Na₂SO₃) was added followed by 7.0ml of Ammonium Molybdate Solution (1.89mM Ammonium Molybdate, acidified conc. H₂SO₄). Thereafter, samples were heated at 100°C for 20 min. Upon cooling, UV absorbance at 830nm was measured and compared against a standard curve of known amounts of phosphates.

Cloud Point Assay: Irinotecan Concentration Determination.
To each 100μl of the eluted samples, 900μl of 1 % Triton X-100 was added. Samples were heated in a boiling water bath for 2 min. After cooling, UV absorbance was measured at 370nm and compared against standards.

Drug Release Studies
Samples were prepared (final lipid concentration 1μmol/ml) and an equal volume of FBS was added. Samples were incubated at 37°C. 100μl aliquots of the samples were collected at various time points. Eluted samples were analysed to determine D/L.

Drug Stability Studies
Within 4 weeks after drug loading, 100μl aliquots of the samples were collected at various time points. Eluted samples were analysed to determine D/L. Size of the liposomes was also monitored using the Zetasizer.
RESULTS & DISCUSSION

Irinotecan Loading Efficiency

As displayed in Fig. 1, the extent and rate of drug loading in liposomes with the same D/L were comparable regardless of the presence of Saf. Rate of irinotecan encapsulation was only limited by the initial drug concentration as liposomes with D/L 0.025 had a slower rate of drug uptake than those with D/L 0.1. Nevertheless, drug loading efficiency of ~100% was achieved.

Drug Release Studies

Figure 2. Rate and Extent of Irinotecan encapsulation under simulated physiological drug release conditions
Drug release was monitored over 24 h. As displayed in Fig. 2, rate of drug release in all formulations reached a peak ~ 0.5-1 h after FBS was added and thereafter decreased. Maximum drug release was attained about 3-5 h earlier in liposomes without Saf. Overall, rate and extent of drug release was greater in liposomes with Saf at D/L 0.1 but lower at D/L 0.025.

Drug Stability Studies

As displayed in Fig 3, overall drug stability of liposomes with and without Saf remained comparable. Stability of the liposomes was not affected by different D/L. Over the 4 weeks of monitoring, there was minimal drug leakage from the liposomes.

CONCLUSION

From the results obtained, liposomes with Saf displayed comparable drug encapsulation efficiency as well as drug stability levels as those without Saf. More importantly, Saf enhanced the efficiency of drug release and this can potentially increase therapeutic efficacy. Overall, it can be concluded that Safingol can be successful co-delivered together with Irinotecan so as to produce stable liposomes with the potential to enhance cytotoxic activity against cancer cells when administered.

REFERENCES