A Novel Approach Towards Formulation Optimization of Vinorelbine PAMAM Dendrimer Conjugates Loading in Liposomal Formulation

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Abstract - The antitumor activity of vinorelbine is may be due to primarily inhibition of mitosis during metaphase and its interaction with tubulin. The aqueous solubility of the drug is > 1000 mg/mL in distilled water. Prolonged vinorelbine exposure is correlated with improved antineoplastic effects, as evidenced by increased response rate in patients receiving continuous infusion. A phase I pharmacokinetic study of the Vinorelbine liposomal injection has been reported and concluded well tolerated and exhibited more favourable pharmacokinetic profiles than free vinorelbine. The Dendrimers are new class of artificial macromolecules. Dendrimer possess a well-defined topological structure, dendrimer through active targeting, and can be accumulated in tumours via the enhanced permeability and retention (EPR) effect of the nanosized dendrimer through passive targeting effect of the nanosized are versatile candidates as scaffolds or vehicles for nanomedicine the field of cancer diagnosis and therapy. Dendrimers can be used for conjugating of anticancer compounds and by non-covalent interactions (ionic, hydrophobic, hydrogen-bond interactions), covalent bindings, and spacer-mediated conjugates. Also, they can be used for targeting to cancer cells, tumour tissues, or abnormal vessels adjacent to the disease focus based on the molecular “hooks” conjugated on the surface of dendrimer through passive targeting. In this study we developed an optimized technology to load PAMAM dendrimers conjugate Vinorelbine to Liposome for improved release and better stability.

Keywords - Vinorelbine, PAMAM dendrimer, Liposome, Cancer, Drug delivery system

1. INTRODUCTION

Vinorelbine is a cytostatic antineoplastic drug. It is a semi-synthetic vinca alkaloid family that interferes with microtubule assembly. The antitumor activity of vinorelbine is due to primarily inhibition of mitosis during metaphase and its interaction with tubulin. In intact tectal plates from mouse embryos, vinorelbine, vincristine and vinblastine inhibited mitotic microtubule formation at the same concentration 2 micro mole, including a blockade of cells at metaphase. The aqueous solubility of the drug is > 1000 mg/mL in distilled water. Prolonged vinorelbine exposure is correlated with improved antineoplastic effects, as evidenced by increased response rate in patients receiving continuous infusion.

Work has already been executed and published on administration of slow release pegylated liposomal vinorelbine formulation by Li CL et al. 2010. A phase I pharmacokinetic study of the Vinorelbine liposomal injection has been reported and concluded well tolerated and exhibited more favorable pharmacokinetic profiles than free vinorelbine. Dendrimers are new class of artificial macromolecules. Dendrimers posses a well – defined topological structure are versatile candidates as scaffolds or vehicles for nanomedicine in the field of cancer diagnosis and therapy. Dendrimers consists of three parts from the interior to the surface: a central core with more than one reactive group, secondly, repeated some units that covalently attached to the central core and organized in a series of radially homocentric layers called generations. Finally, peripheral functional groups existed on the surface which majorly determines the physicochemical properties. Dendrimers can be used for conjugating of anticancer compounds and/or diagnostic probes by non-covalent interactions (ionic, hydrophobic, hydrogen-bond interactions), covalent bindings, and spacer-mediated conjugates. Also, they can be used for targeting to cancer cells, tumour tissues, or abnormal vessels adjacent to the disease focus based on the molecular “hooks” conjugated on the surface of dendrimer through active targeting, and can be accumulated in tumours via the enhanced permeability and retention (EPR) effect of the nanosized dendrimer through passive targeting effect of the nanosized are versatile candidates as scaffolds or vehicles for nanomedicine the field of cancer diagnosis and therapy. Dendrimers consists of three parts from the interior to the surface: a central core with more than one reactive group, secondly, repeated some units that covalently attached to the central core and organized in a series of radially homocentric layers called generations. Finally, peripheral functional groups existed on the surface which majorly determines the physicochemical properties. Dendrimers can be used for conjugating of anticancer compounds and/or diagnostic probes by non-covalent interactions (ionic, hydrophobic, hydrogen-bond interactions), covalent bindings, and spacer-mediated conjugates. Also, they can be used for targeting to cancer cells, tumour tissues, or abnormal vessels adjacent to the disease focus based on the molecular “hooks” conjugated on the surface of dendrimer through active targeting, and can be accumulated in tumours via the enhanced permeability and retention (EPR) effect of the nanosized dendrimer through passive targeting effect of the nanosized are versatile candidates as scaffolds or vehicles for nanomedicine the field of cancer diagnosis and therapy. Dendrimers consists of three parts from the interior to the surface: a central core with more than one reactive group, secondly, repeated some units that covalently attached to the central core and organized in a series of radially homocentric layers called generations. Finally, peripheral functional groups existed on the surface which majorly determines the physicochemical properties. Dendrimers can be used for conjugating of anticancer compounds and/or diagnostic probes by non-covalent interactions (ionic, hydrophobic, hydrogen-bond interactions), covalent bindings, and spacer-mediated conjugates. Also, they can be used for targeting to cancer cells, tumour tissues, or abnormal vessels adjacent to the disease focus based on the molecular “hooks” conjugated on the surface of dendrimer through active targeting, and can be accumulated in tumours via the enhanced permeability and retention (EPR) effect of the nanosized dendrimer through passive targeting effect of the nanosized are versatile candidates as
technology to load vinorelbine-PAMAM conjugate in to liposome.

2. MATERIAL AND METHODS

A sample quantity of vinorelbine tartrate has been received from Dr. Reddy’s Laboratories limited on request basis as free sample. Similarly, lipids such as DOPC, DSPC and EPC were procured from Avanti polar lipids. Cholesterol was procured from Avanti polar lipids. PANAM generation 2 were purchased from Sigma Aldrich. Keeping the variability and impurity in the in-house synthesis of PANAM, it was decided to procure from a commercial source. Other chemicals like methanol, sodium 1-decane sulfonate and sodium dihydrogen phosphate for HPLC method establishment were procured from Thermo Fisher. NAVELBINE (vinorelbine tartrate injection) was procured from market. Estimation of Vinorelbine was done using optimized HPLC method for estimation of Vinorelbine was done by referring HPLC method for quantification of vinorelbine discussed in Xiao et al. 2012.

2.1 Conjugation of Vinorelbine to PAMAM G2

G2 PAMAM dendrimer was dissolved in 1 mL of DMSO separately (quantity of PAMAM was 10 mg was taken initially). Vinorelbine was reared at a concentration of 2 mg/mL in DMSO. The drug solution was then added drop-wise to the dendrimer solution at controlled room temperature (20-25°C) with vigorous stirring for 24 hrs. Resulting vinorelbine- PAMAM conjugate was subjected to dialysis using 1000 Da dialysis membrane in distilled water to remove free vinorelbine.

Dialysis membrane was selected based on the molecular weight differences between the vinorelbine and PAMAM dendrimers. The molecular weight of G2 is 3 KDa, whereas the molecular weight of vinorelbine is around 800 Da. Hence, a 1 KDa cut-off membrane will permeate the unbound vinorelbine leaving behind the conjugated drug.

2.2 Optimization of Liposome preparation

It was reported in the literature that, the major function of cholesterol is on the permeability of liposomes, cellular uptake, internalization mechanisms, and cytotoxicity. The fluidity of a lipid bilayers changes with temperature. The lipid bilayer exists in a solid (gel) phase at below the phase transition temperature (Tm) and exists in the liquid phase at the temperature above Tm. In the solid (gel) phase, lipids are packed tightly and the lipid bilayers exhibit a high stability. In the liquid phase, on the other hand, lipids exchange their location with their adjoining lipids millions of times a second and this enhances permeability of bilayers. However, it has been known that the presence of cholesterol induces the stability of liposomes since cholesterol molecules fill in the free space between lipids as a large number of studies have shown. Hence, cholesterol is having importance in stability of liposomes. In this formulation lipid such as pegylated DSPE has been selected with cholesterol with various proportions such as 75:25, 50:50 and 25:75 respectively. Liposome has been prepared by using film hydration method using rotary evaporator. First, Lipid was weighed and added to a mixture of 4:1 proportion of DCM and methanol in a round bottomed flask. The flask was connected to a rotary evaporator and the solvent was evaporated at 60°C till complete drying. It took around the 30 minutes for complete evaporation.

Vinorelbine -PAMAM conjugate prepared (optimized previously in Samantray et al., was re-suspend in double distilled water using solvent exchange method. Then Vinorelbine - PAMAM conjugate in double distilled water was used to hydrate the dried lipid film followed by vigorous shaking to form multi lamellar vesicles (MLVs). MLVs were then subjected to sonication for 60 min and then pass through 100 nm polycarbonate membrane for extrusion using Lipofast Pneumatic extruder. The resulting unilamellar liposome suspension was centrifuged at 25,000 rpm for 30 min to remove residual Dendrimers. The pellet was re-suspended in 1 ml of 8 % sucrose and lyophilized over 72hrs.

2.3 Optimization of Vinorelbine-PAMAM conjugates concentration for optimum loading

A stock solution of 20 mg/mL of Vinorelbine-PAMAM G2 conjugate was diluted with double distilled water based on the concentration of vinorelbine such as 2 mg/mL, 5 mg/mL, 8 mg/mL, 10mg/mL, and 20 mg/mL. Prepared conjugate solution was separated in five different glass tubes and labelled properly then subjected to encapsulation in to liposome using a cholesterol and lipid ratio of 75:25. Then Vinorelbine -PAMAM conjugate in double distilled water was used to hydrate the dried lipid film followed by vigorous shaking to form multi lamellar vesicles (MLVs). MLVs were then subjected to sonication for 60 min and then pass through 100 nm polycarbonate membrane for extrusion using Lipofast Pneumatic extruder. The resulting unilamellar liposome suspension was centrifuged at 25,000 rpm for 30 min to remove residual dendrimers. The supernatant was collected for all dilution and subjected to HPLC analysis for Vinorelbine using the optimized method discussed in Samantray et al.

2.4 Optimized Process of Loading

A solution of 10 mg/mL of Vinorelbine-PAMAM G2 conjugates was prepared in double distilled water. Lipid such as pegylated DSPE has been taken with cholesterol with a proportion cholesterol and lipid ratio of 75:25. Liposome has been prepared by using film hydration method using rotary evaporator.Vinorelbine –PAMAM conjugate prepared 10 mg/ mL was re-suspend in double
distilled water. Then Vinorelbine –PAMAM conjugate in double distilled water was used to hydrate the dried lipid film followed by vigorous shaking to form multi lamellar vesicles (MLVs). MLVs were then subjected to sonication for 60 min and then pass through 100 nm polycarbonate membrane for extrusion using Lipofast Pneumatic extruder. The resulting unilamellar liposome suspension was centrifuged at 25,000 rpm for 30 min to remove residual dendrimers. The pellet was re-suspended in 1 mL of 8 % sucrose and lyophilized over 72 hrs. Release study was performed by dialysis, 3 ml of reconstituted liposomal formulation with optimum drug to lipid ratio, as explained earlier, was transferred into dialysis membrane tubing, which was then placed into 300 ml of dialysis medium (PBS buffer, pH 7.4) at 37oC under constant slow stirring and kept in dark throughout the experiment. Two mL of dialysis medium were withdrawn at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 20, 22, 28, and 48 h of the experiment. Samples were also taken from the dialysis membrane tubing before and after the experiment. Release studies were conducted for 48 h without any change or replacement of dialysis medium.

3. RESULTS AND DISCUSSION

Encapsulated Vinorelbine -PAMAM conjugate prepared with different proportion of cholesterol and lipid such as 25:75, 50:50 and 75:25 were reconstituted with phosphate buffer saline and kept for 30 minutes. Then the content was centrifuged at 25,000 rpm for 30 min. The supernatant of each tube were collected and subjected to HPLC analysis of Vinorelbine using the optimized method discussed earlier.

It was found that, 75:25 proportion of cholesterol and lipid shows a high loading efficacy. Therefore, the same proportion was used for optimization of conjugate concentration.

Figure 1: Loading efficiency of Vinorelbine-PG2 Conjugate

It was observed that, 10 mg/ml concentration the % loading was 90%. At 20 mg/ml concentration the % loaded drug was decreased to 88.0%. Therefore, we found the maximum loading capacity of the 10 mg/ml Vinorelbine-Pamam G2 conjugate.

Figure 2: Effect of Vinorelbine-Pamam G2

It was observed from the comparative release study that, drug release from Pamam conjugated one is delayed than that of the un-conjugated drug liposomal formulation. From the above we have summarized that, the formulation, as release characteristics of the Pamam conjugated vinorelbine is superior to the un-conjugated vinorelbine in liposomal formulation.

4. CONCLUSION

PAMAM dendrimer has been proven its safety previously and has been used as for human consumption for parenteral route. US-FDA also approved them as a diagnostic device for quantification of NTproBNP in human plasma. In this research work, we have described the optimized drug conjugated liposomal formulation has improved release of an anti-cancer drug Vinorelbine & shown a improved release kinetics than the unconjugated liposomal formulation. The release kinetics of the drug can be further modified by some advance encapsulation of the conjugate. Furthermore, the therapeutic dendrimer based nano particulate formulations are under approval process.

For human use by US-FDA We believe the findings published in this article will enlighten the new
dimensions for modified release dosage form in the field of oncotherapeutics.

REFERENCES


