

Original Article

An approach towards optimized dendrimer based delivery technology to modify drug loading and release of Vinorelbine

Satyaswarup Samantray^{a,*}, Sudhansu R. Swain^b, Shailesh Sharma^c.

^a*Mylan Laboratories Ltd. R&D Center, Bollaram, Hyderabad, Andhra Pradesh, India.*

^b*Moradabad Educational Trust, Group of Institutions - Faculty of Pharmacy, Moradabad, Uttar Pradesh, India.*

^c*Department of Pharmaceutics, NIMS Institute of Pharmacy, NIMS University, Jaipur, Rajasthan, India.*

* *Corresponding Author: Tel.: +91 7702686117; E-mail address: pulin83@gmail.com*

ARTICLE INFO

Received 22 Jul 2017

Revised 12 Aug 2017

Accepted 16 Aug 2017

Keywords:

- Vinorelbine
- PAMAM dendrimer
- Cancer
- Drug delivery system

ABSTRACT

The antitumor activity of vinorelbine 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 favorable pharmacokinetic profiles than free vinorelbine. Dendrimers are new class of artificial macromolecules. Dendrimers possess a well-defined topological structure, are versatile candidates as scaffolds or vehicles for nanomedicine in the field of cancer diagnosis and therapy. Dendrimers can be used for loading 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 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 dendrimer through passive targeting. In this study we developed an optimized technology to conjugate Vinorelbine to PAMAM dendrimers for improved release and better stability than the liposomal 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 may be due to primarily inhibition of mitosis during metaphase and its interaction with tubulin [1]. 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 [2-

5]. 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 [6]. 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 [7-9]. Dendrimers are new class of artificial macromolecules. Dendrimers possess a well-defined topological structure, are versatile candidates as scaffolds or vehicles for nanomedicine in the field of cancer diagnosis and therapy [10-12]. 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 [13]. Dendrimers can be used for loading of anticancer compounds and/or diagnostic probes by non-covalent interactions (ionic, hydrophobic, hydrogen-bond interactions), covalent bindings, and spacer-mediated conjugates [14-15]. 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 dendrimer through passive targeting. In this study we developed an optimized technology [16].

2. EXPERIMENTAL

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 1 and 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-decanesulfonate and sodium dihydrogen phosphate for HPLC method establishment were procured from Thermo Fisher. NAVELBINE (vinorelbine tartrate injection) was procured from market

2.1 Optimization of HPLC method for estimation of Vinorelbine

HPLC method for quantification of vinorelbine tartrate has been discussed in Xiao-hong et al. 2012 [15]. Here the same method was established and optimized to analyze the sample. Presently we do not have optimized final formulation so the optimization of HPLC method has been carried out using marketed formulation and standard API of Vinorelbine Tartrate (Fig 1). However the optimized analytical method will be confirmed with final formulation.

Optimized chromatographic parameters are as follows

- Column: Supecasil ABZ+Plus C18
- Mobile phase: 0.05 mol·L⁻¹ sodium dihydrogen phosphate solution (adjusted to pH 4.2 with phosphate acid)-0.2% sodium 1-decanesulfonate solution in methanol (40:60).

- Diluent: Mobile phase
- Flow Rate: 1 mL·min⁻¹
- Detection Wavelength: 267nm
- Temperature: 35°C

Reported literature says that, with increase in the generation, branching of the PAMAM also increases and simultaneously drug loading capacity also increases. But with increase in the generation the toxicity of the PAMAM also increases and it is considered that after G3 it is not recommended for human use. Hence, in this study only G1 and G2 are included and a comparative loading efficiency was demonstrated (Fig 2). The loading efficiency was determined by performing dialysis after the conjugation reaction.

A fixed quantity of PAMAM G2 (Sigma Aldrich) dendrimer 10 mg was dissolve in 1 mL of DMSO (Sigma Aldrich). In a different glass tube four different concentration of vinorelbine was prepared in DMSO (Sigma Aldrich) such as 5 mg/mL, 10 mg/mL, 15 mg/mL, 20 mg/mL and 25 mg/mL. These concentrations were selected based on the concentration of dendrimer, which is 10 mg/mL (Fig 3).

Prepared dendrimer solution was separated in five different glass tubes and labeled properly. Then vinorelbine solution was added to the respective labeled dendrimer solution separately drop wise. During addition the reaction mixture was stirred vigorously. The above reaction was kept for 24 hrs at room temperature with stirring.

A three different preparation was done by adding 10 mg of PAMAM G2 dendrimer in 1 mL of DMSO. In a separate glass vessel drug solution was prepared with the above optimized concentration of 20 mg/mL as three separate preparations. The above prepared PAMAM solution was labeled properly as 12 hrs, 24 hrs and 48 hrs as per the incubation time. Then the drug solution was added drop wise with vigorous stirring. It was then kept for 12 hrs, 24 hrs and 48 hrs as per the labeled reaction vessels. After completion of the incubation the solution was subjected to dialysis with 1 KDa membrane using distilled water for 3 days. On completion of dialysis the distilled water sample was analyzed for vinorelbine by HPLC (Fig 4).

For optimization of reaction temperature, two different reaction temperatures was selected, such as 5±3°C, 20-25°C and 30-35°C. The above two temperature conditions were obtained by a water bath with provision of thermostat, a heating element and a chiller for circulation of chilled water. The temperature of the reaction vessel was monitored by a digital thermometer. In this the above optimized concentration of dendrimer solution and vinorelbine drug solution was taken in two reaction vessel. The addition of drug solution and throughout the reaction was carried out at respective temperature (Fig 5).

2.2 Conjugation of Vinorelbine to PAMAM G2

G2 PAMAM dendrimers 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 PAMAM G1 and G2 are 1.5KDa and 3 KDa respectively, 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.

2.3 Procedure for release study

Release study was carried out by dialysis method. 3 mL of formulation was injected into dialysis membrane tubing (Size cut off: 1 KDa) it was placed in to 300 mL of phosphate buffer saline (pH 7.4) at 37°C with continuous stirring. Two mL of dialysis medium were withdrawn at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 20, 24, 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 (Fig 6).

3. RESULTS AND DISCUSSION

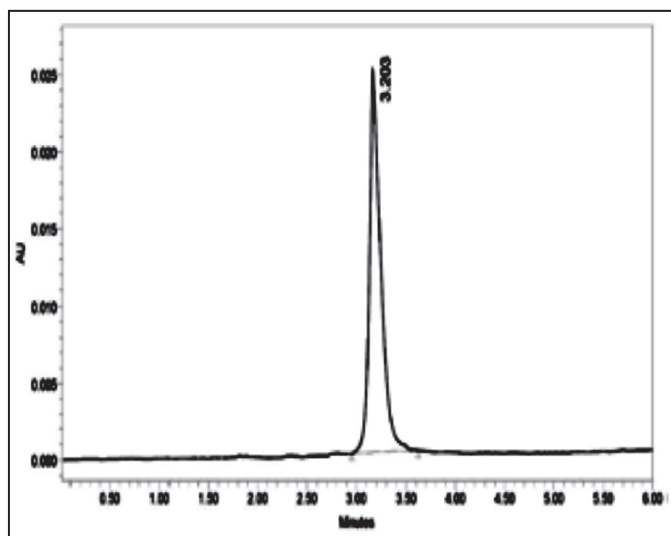


Fig. 1. Typical chromatographic peak of Vinorelbine

Conjugation of vinorelbine and PAMAM of G1 and PAMAM G2 was performed separately by incubating the DMSO (Sigma Aldrich) solution of vinorelbine and PAMAM dendrimers. Dialysis was performed using 1 KDa cut-off membrane (GE healthcare) and distilled water. Free vinorelbine dialyzed out from the dialysis membrane was estimated using optimized assay method.

Further the parameters like incubation time temperature of the conjugation reaction, stirring speed of the reaction are also optimized and results are as follows:

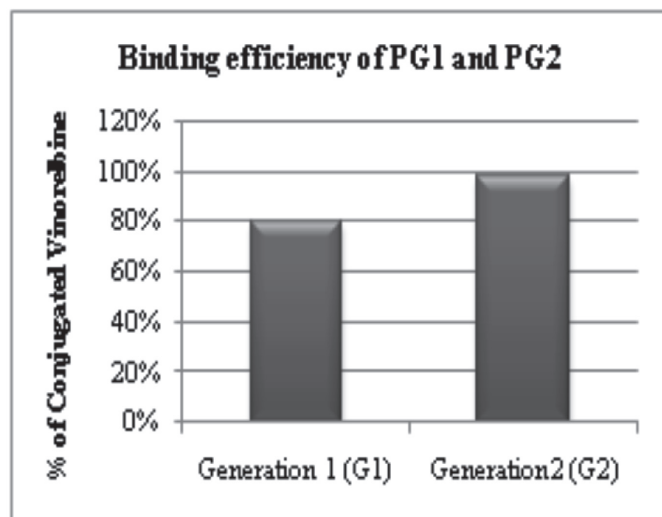


Fig. 2. Binding efficiency of PG1 and PG2

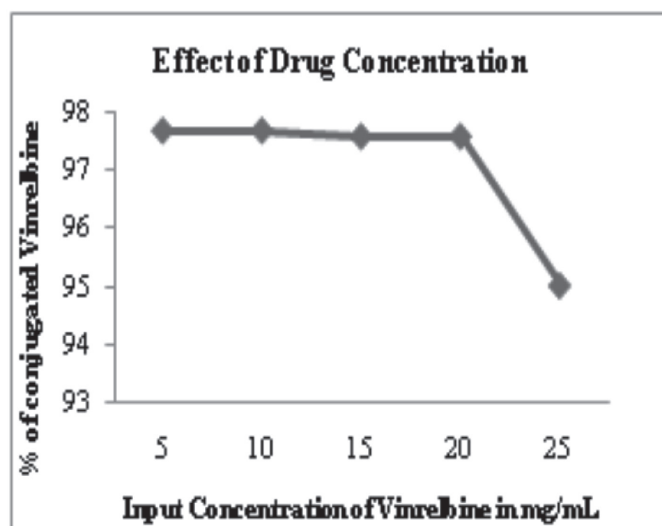


Fig. 3. Effect of drug concentration on percentage of conjugated Vinorelbine

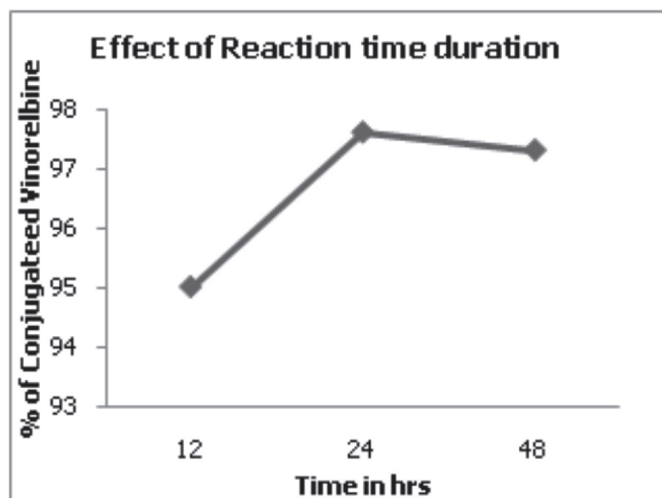


Fig. 4. Effect of reaction time duration on percentage of conjugated Vinorelbine

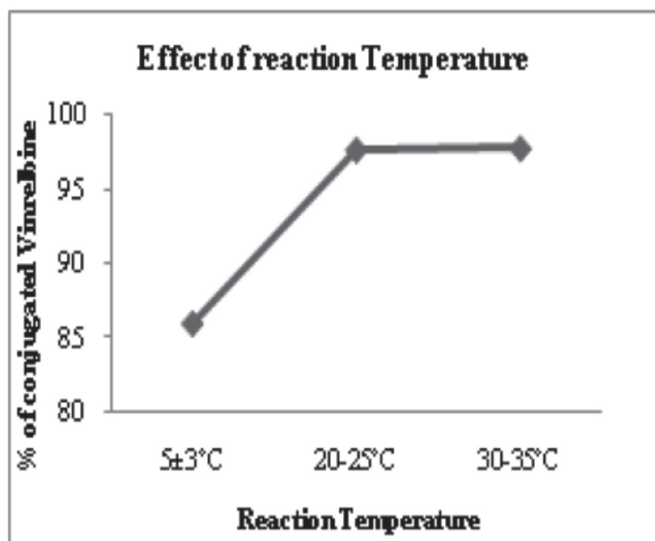


Fig. 5. Effect of reaction temperature on percentage of conjugated Vinorelbine

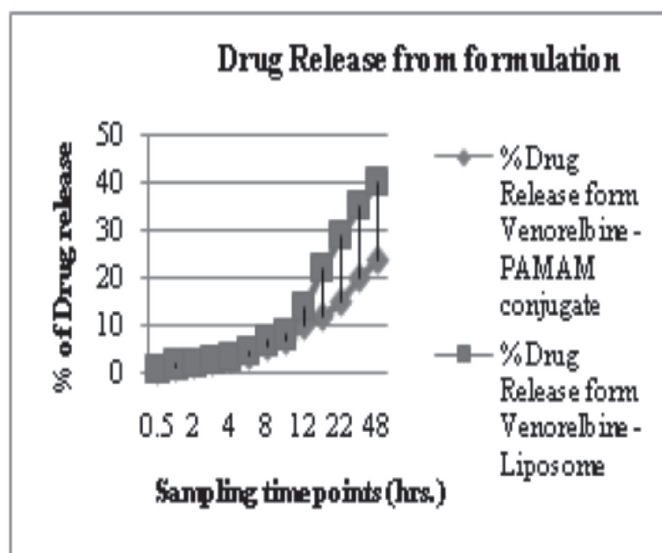


Fig. 6. Percentage of drug released from Vinorelbine formulation

Above results shows that PAMAM G2 (PG2) is having more conjugation efficiency due to higher branching. But we can't go further generation due to toxicity. Based the above optimized parameter a formulation of Vinorelbine-PAMAM G2 was prepared and the release of vinorelbine was compared with the liposomal formulation of vinorelbine. Compared to liposomal formulation the release of drug is prolonging in case of Vinorelbine-PAMAM G2 formulation.

4. CONCLUSION

PAMAM dendrimers 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 (Anupa R. M, *et al.*). Here we

have described the optimized conjugation and improved release of an anti-cancer drug Vinorelbine, which shown a better release than the 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. Hope the findings published in this article may enlighten the new dimensions for modified release dosage form in the field of onco therapeutics.

REFERENCES

- [1] Anupa RM, Rangaramanujam MK, Donald AT. Dendrimer-based drug and imaging conjugates: design considerations for nanomedical applications. *Drug Discovery Today* 2010; 55 (6): 171-185.
- [2] Bardhan R, Lal S, Joshi A, Halas NJ. Theranostic nanoshells: from probe design to imaging and treatment of cancer. *Acc Chem Res.* 2011; 44: 936-946.
- [3] Bhattacharya S, Kudgus RA, Bhattacharya R, Muherjee P. Inorganic nanoparticles in cancer therapy. *Pharm Res.* 2011; 18: 237-259.
- [4] Bushman J, Vaughan A, Sheihet L, *et al.* Functionalized nanospheres for targeted delivery of paclitaxel. *J Control Release.* doi: 10.1016/j.jconrel.2013.06.017.
- [5] Costas D, Natassa P. Advanced drug delivery nanosystems (aDDnSs): a mini-review. *Drug Deliv. Online.* 2013: 1-8.
- [6] Drummond C, Charles O, Zexiong N, Mark EH, John WP, Ching-JO, Yun-Long T, Keelung H, Dmitri BK. Improved Pharmacokinetics and Efficacy of a Highly Stable Nanoliposomal Vinorelbine. *J Pharmacol Expt Ther.* 2008; 320(1): 321-330.
- [7] Gardikis K, Hatziantoniou S, Bucos M, Fessas D, Signorelli M, Felekis T, Zervou M, Screttas CGS, Steele BR, Ionov M. New drug delivery nanosystem combining liposomal and dendrimeric technology (liposomal locked-in Dendrimers) for cancer therapy. *J. Pharm. Sci.* 2010b; 99: 3561–3571.
- [8] Gardikis K, Hatziantoniou S, Signorelli M, Pusceddu M, Michascrettas M, Schiraldi A, Demetzos C, Fessas D. Thermodynamic and structural characterization of liposomal-locked in-dendrimers as drug carriers. *Colloids Surf. B: Biointerfaces.* 2010; 81: 11–19.
- [9] Gardikis K, Hatziantoniou S, Viras K, Wagner M, Demetzos C. A DSC and Raman spectroscopy study on the effect of PAMAM dendrimer on DPPC model lipid membranes. *Int. J. Pharm.* 2006; 318: 118–123.
- [10] James R, Baker Jr. Dendrimer-based nanoparticles for cancer therapy. *Hematol.* 2009; 708-719.
- [11] Kaditi E, Mountrichas G, Pispas S, Demetzos C. Block Copolymers for Drug Delivery Nano Systems (DDnSs). *Cur Med Chem.* 2012; 19: 5088-5100.

- [12] Khopade AJ, Caruso F, Tripathi P, et al. Effect of dendrimer on entrapment and release of bioactive from liposomes. *Int J Pharm* 2002; 232: 157–62.
- [13] Konstantinos G, Chriiida T, Konstantinos D, Maria MS, Costas D. New chimeric advanced Drug Delivery nano Systems (chi-aDDnSs) as doxorubicin carriers. *Int J Pharm*. 2010; 402: 231-237.
- [14] Papagiannaros A, Dimas K, Papaioannou G, Demetzos C. Doxorubicin-Pamam dendrimer complex attached to liposomes and cytotoxic studies against cancer cell lines. *Int J Pharm* 2005; 302: 29–38.
- [15] Xia H, Tang W, Yin Q, Zhang B, Wang Dong-H, Yang Q. HPLC determination of vinorelbine tartrate in liposome-encapsulated injection. *J Pharm. Analysis* 2012; 32(12): 1882-1885.
- [16] Li CL, Cui JX, Wang CX, Zhang L, Li YH, Zhang L, Xiu X, Li YF, Wei N. Development of pegylated liposomal vinorelbine formulation using «post-insertion» technology. *Int. J Pharm*. 2010; 91(1-2): 230-236.