Available online at www.elixirpublishers.com (Elixir International Journal)

Pharmacy

Elixir Pharmacy 38 (2011) 4118-4121

ISSN: 2229-712X

Development and performance evaluation of multilayered Nanoparticles for delivery of Docetaxel

Manju Nagpal[1], Naresh Kalra[1], G.Jeyabalan[1], Narender Kumar Noyla[2] and Pankaj Rakha[3]

[1]Department of Pharmaceutics, Alwar Pharmacy College, Alwar.

[2]Department of Pharmaceutics, Chitkara School of Pharmaceutical Sciences, Chitkara University, Barotiwala (HP)

[3]Department of Pharmacology, Rajendra Institute of Technology & Sciences, India.

ARTICLE INFO

Article history:

Received: 24 June 2011;

Received in revised form:

22 August 2011;

Accepted: 27 August 2011;

Keywords

Liposomes,

Chitosan nanoparticles,

Docetaxel,

Entrapment efficiency,

In vitro release,

Ethanol injection.

ABSTRACT

Liposomes are an important colloidal carrier system for controlled drug delivery. However some highly hydrophilic small molecules are difficult to entrap into liposomes and store stably, resulting in poor encapsulation efficiency and fast leakage. In present study, Docetaxel was used as a model drug that was loaded into chitosan nanoparticles and the encapsulated into liposomes by ethanol injection method (EIM). The vesicular systems were characterized for particle size, zeta potential, Transmission Electron Microscopy (TEM) and evaluated for encapsulation efficiency and in *vitro* release. The Lip-Np was composed of Hydrogenated Soya phosphatidylcholine, Cholesterol, EPG and Chitosan with average diameter of 207.8nm and zeta potential of +21.7mv. The entrapment efficiency was above 90% in Chitosan coated (Lip-Np). The release rate of docetaxel from Chitosan coated Lip-Np was more than 90% after 72h.

Introduction

Liposomes are one of the most widely used drug delivery systems, having been investigated for delivery of chemotherapeutic agents for cancer. Controlled release is one of the most important elements in the design of liposomes for the delivery of its contents. The release of drug depends not only on the composition of lipid bilayer, but also on interaction of liposomes with tissue and biological fluids. The application of liposomes given systemically is limited, due to their rapid clearance by endothelial system. Further multilamellar vesicles made by classical methods have yielded structures which entrapped minimal quantities of drug, are extremely unstable in biological fluids, and are recognized and cleared rapidly by macrophages. When drugs are incorporated into the liposome, consideration must be given to prevent leaking and loss of drug through the membrane[1].

Docetaxel (DTX) is more potent inhibitor of microtubules depolymerisation as compared to Paclitaxel[2-3]. DTX is a natural product with anti-tumor activity. Taxol is obtained via a semi-synthetic process from Taxus baccata[5-6]. Docetaxel belongs to taxane category of antineoplastic agents, and most important chemotherapeutic agents against cancer[7-11].

In this present study, chitosan coated liposomal drug delivery system was developed for intravenous administration of DTX by "Ethanol injection method, able to improve the drug solubility [4]. The vesicular systems were characterized for size distribution Zeta potential study and surface chemistry and evaluated for drug encapsulation efficiency and *in- vitro* release.

Material and Methods

Material

Egg phosphatidylglycerol and Cholesterol were purchased from Avanti Polar Lipids (AL, USA) and HSPC from AstaMedica/Baxter (Bielefeld, Germany). Chitosan was obtained from central Institute of Fisheries Technology, Kochi. Docetaxel was kindly gifted from Dabar Ltd. All the other chemicals and solvents were of analytical reagent grade. Deionised Double- distilled water was used throughout the study.

Preformulation investigation

Determination of Solubility

The approximate solubility of docetaxel in different aqueous and non-aqueous media (including distilled water, methanol, acetonitrile, ethanol and soya been oil) was determined. Equivalent amount of the drug was added and shaken in separate flask containing different solvents using a mechanical shaker at room temperature (25°C) for 24 hrs. The samples were then centrifuged at 2000 rpm, filtered through membrane filter and analyzed by HPLC to determine the amount of Docetaxel dissolved.

Determination of Partition Coefficient

The partition coefficient between octanol/water was determined at room temperature (30°C). Ten mL of octanol and 10 mL of distilled water were taken in a glass stopper graduated tube and 5 mg of accurately weighed drug was added. The mixture was then shaken using mechanical shaker for 24 hrs at room temperature. The mixture was then transferred to a separating funnel and allowed to equilibrate for 6 hrs. The aqueous and octanol phase were separated and filtered through membrane filter and drug content in aqueous phase was analyzed by HPLC. The apparent partition coefficient was obtained by the ratio of docetaxel concentration in octanol phase to aqueous phase. The partition coefficient of the drug was determined in two systems i.e. in n-Octanol/water and in Isopropylmyristate/water. The partition coefficient was calculated by following formula:

Partition coefficient (PC) = C_t-C_a/C_a

Tele:

E-mail addresses: neshu07@gmail.com

Where C_t is the concentration of the total drug taken

C_a is the concentration of the drug in aqueous phase

Preparation of Liposomes-Encapsulating Chitosan Nanoparticles (Lip-Np)

Lip-Np was prepared by Ethanol injection method. An ethanolic solution containing 19 mg of hydrogenated Soya phosphatidylcholine (HSPC), 7.5 mg of cholesterol, 6 mg EPG and 6.5 mg docetaxel. 0.02% solution of chitosan was prepared separately. Then this lipid drug solution was injected slowly over 5 minutes through a syringe pump using a gauge needle into rapidly stirred 2 ml of chitosan solution. The system was then stirred continuously for 3 minutes. The equilibrated liposomal suspension was transferred to an Amicon ultra filtration apparatus where it was filtered through an ultra filtration membrane. Analysis of the filtrate using HPLC method described previously revealed an encapsulation efficiency of docetaxel in the formulation.

Encapsulation Efficiency Analysis

Gel Exclusion Chromatography was employed to determine entrapment efficiency. The formulation was passed through Sephadex G-100 column to separate unentrapped drug. The suspension of vesicular formulations was then centrifuged at 2000 rpm for 3 minutes. The liposome formulations were analyzed drug content was estimated by HPLC. Entrapment efficiency was used as a parameter in selecting the type of lipid, lipid and chitosan ratio amount of drug encapsulated in vesicular formulation[13-14].

% Entrapment = {(Total amount of drug- Free drug)/ Total amount of drug} ×100

Size and Polydispersity Index (PdI) measurements

The mean particle size & particle size distribution of vesicles and drug loaded vesicles were determined by a Malvern zetasizer NanoZS (Malvern 3000HS, France). Each sample was measured in triplicate. The Polydispersity Index (PI) was calculated by using an equation standard deviation/size of vesicles[15-16].

Zeta Potential

Electrophoretic mobility of liposome (plain and docetaxel loaded) were measured using a Malvern zetasizer NanoZS (Malvern 3000HS, France). Optical properties of the sample were defined as follows: refractive index 1.460 and absorption 0.00. The mobility u was converted into zeta potential (ξ) values using the Smoluchowski relation $\xi = u\eta/\in$; where η and $\in$ are the viscosity and permittivity of the solution, respectively[17]. All ξ-potential measurements were performed without added electrolyte. Finally the data of optimized formulations was recorded. In general, particle aggregation is less likely to occur for charged particles (high zeta potential) due to electric repulsion [18-19].

Transmission electron microscopy (TEM)

A drop of the liposome suspension was placed on carbon-coated grid, dried in the air for 15 min and negatively stained with 1.0% uranyl acetate (UA) solution. Excess UA was washed with 50% aqueous ethanol followed by double- distilled water, air- dried and examined in Philips CM 12 EM. The accelerating voltage applied was 60 kV[20].

***In-vitro* drug release studies**

The *in-vitro* docetaxel release profile from optimized formulations was determined using artificial dialysis bag (Sigma, Cut off mwt. 12000 Dalton)[21].Two ml of each of pure docetaxel solution and liposome formulation of different drug concentration was taken into a pretreated dialysis bag and placed in baskets of dissolution assembly containing 200 ml of triple distilled water. The stirring speed was kept at 50 rpm and the temperature of the assembly was maintained at 37± 1°C throughout the study. At appropriate intervals, all receptor medium were withdrawn and immediately replaced by an equal volume of fresh receptor solution (TDW). The withdrawn samples were analyzed for drug by HPLC[22].

Result and Discussion

Preformulation investigation

Four samples of 100mg docetaxel completely dissolved in 0.1 ml methanol, ethanol, distilled water, soya been oil respectively. It suggested that Docetaxel is very soluble dug in methanol, ethanol, acetonitrile and slightly soluble in soya been oil and insoluble in distilled water. The partition coefficient was found out to be 1.35 in n-octanol: water and 1.46 in Isopropylmyristate/water. This concludes that drug is amphiphilic behaviour, since the drug did not show any special preference between water and the organic solvents studied

Preparation of (Lip-Np) by EIM

Several methods have been developed for preparation of Lip-Np including thin-film hydration, RPV techniques and ethanol injection method. However the Lip-Np suspension prepared by former two techniques was found to be unstable. A stable and macroscopically homogeneous suspension of Lip-Np was obtained by EIM method.

High Encapsulation Efficiency obtained by Lip-Np using EIM

The encapsulation efficiencies of different formulations depend upon ratio of chitosan and lipids. Lower encapsulation efficiency was obtained in plain liposomes. As EPG concentration increase the encapsulation efficiency is more than 90%. It has been observed that these vesicular formulations get stabilized with optimum amount lipids and drug concentration [23-24].

Zeta Potential and Particles Size measurements

The mean particles sizes were 251.6, 207.8 and 318.6nm & the value of PdI is 0.478, 0.586 and 0.486 for varing concentration of HSPC, EPG and constant concentration Cholesterol. Chitosan concentration of 0.02% is selected for the smaller particle size of vesicles

Zeta potential has been used for characterizing colloidal drug delivery systems and these measurements facilitate the understanding of the dispersion and aggregation processes. The zeta potential of Lip-Np encapsulating Chitosan was higher than blank liposomes. This difference may be attributed to influence of positively charged cores. Charge of the core and lipid layer may neutralize, which results in increasing of zeta potential. Mixtures of Chitosan liposomes, blank liposomes, oppositely charged, neutralize each other and cause aggregation and fusion. This results in increased particles size and neutralizes zeta potential.

The size, shape and structure Plain Liposomes and Chitosan coated liposomes were studied by TEM. Figure 1 shows a transmission electron micrograph of Plain liposomes. The spherical structure of liposomes coated with chitosan was confirmed by TEM Figure 2. The vesicles size was found to be 100-200

***In vitro* drug release studies**

In-vitro release profile of various DTX formulations (LP1 and LP2) has been represented in figure 3 It has observed that LP2 released 93.6% after 72 hours of study as compared to LP1 formulation. This data indicates that incorporation of cholesterol

above phase transition temperature made the bilayer more ordered resulting in slow release.

Fig. 1 Transmission electron micrograph of blank liposomes stained with 1% urenyl acetate

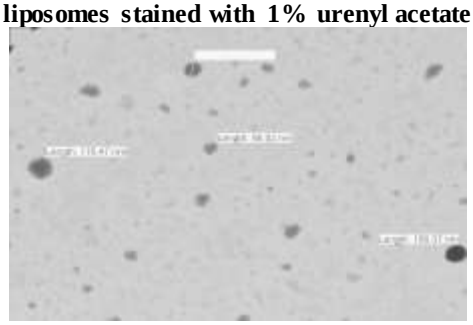

Fig.2 Transmission electron micrograph of chitosan coated liposomes stained with 2% urenylacetate and 1% lead citrate

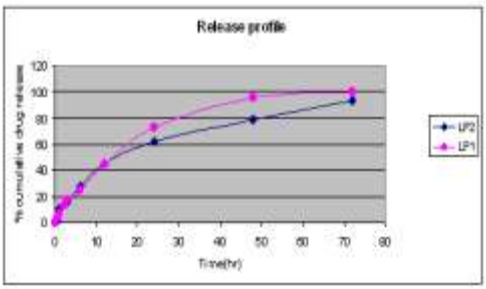

Fig. 3 Drug release profile of study of LP1 and LP2. The errors bars indicates S.D of three set of experiments (n= 3).

Conclusion

From the all above study, it can be concluded that the liposomes formulation represents better sustained drug delivery system for cancer. Preformulation investigation suggests Docetaxel is highly lipophilic and insoluble in water. The liposomes prepared by ethanol injection method yield high entrapment efficiency due to high interaction with lipids. The major advantages of lipid matrix without complicated chemistry or hazardous procedures. Scientists from different fields must combine forces to work on liposomes improving their stability and exploring their toxicological and immunological properties. Liposomes encapsulating a solid core exhibit excellent potential both *in vitro* and *in vivo* for drug delivery. Chitosan used as core material is advantageous to achieve high drug entrapment efficiency due to oppositely charged to lipid layer as shown in this work.

References

1. Uchegbu, I. F., Bouwstra, J. and Florence, A. T (1992) J. Pharm. Pharmacology. (Suppl.), 1052.

2. Gueritee-voegelein F, Guenard D, Lavelle F,Le Goff MT.Mangatal L; Potier P(1991) Relationships between the structure of Taxol analogues and their antimitotic activity's Med.Chem 34:992-998.

3. Zhijiun Yang, David W.F.Fong; Linlin Yin, Yuenfan wong, Wenhua Huang(2009) Liposomes modulate docetaxel- induced lipid oxidation and membrane damage in human hepatoma cells, Journal of Liposome Research, 19: 2, 122-130.

4. Gelderblom, H., Verweij, J., Nooter, K., sparreboom, A. (2001) Cremophor EL: the drawbacks and advantages of Vehicle selection for drug formulation.Eur.J.Cancer 37,1590-1598.

5. Mathew A, Mejillano, Math JP, Hines RH, Stella VJ (1992), Synthesis and evaluation for some water soluble prod rugs and derivatives of Taxol with antitumor activity J. Med Chem, 35, 145-51

6. Herbst, R.S.Khuri, R., (2003). Mode of action of Docetaxel a basis for combination with novel anticancer agents. Cancer Treat. Rev.29, 407-415.

7 .R. H. Earhart (1999), Docetaxel (Taxotere): Preclinical and general clinical information, Semin, Oncol.26 (5) 8-13.

8. E. K.Rowinsky, R.C.Donehower, Paclitaxel (Taxol), New Engl(1995) J.Med.332 1004-1092.

9. E.K.Rowinsky (1997) The development and clinical utility of the taxane class of antimicrotubule chemotherapy agents, Annu. Rev. Med.48 353-374.

10. K.Gelmon (1994), the Taxoids: Paclitaxel and docetaxel, lancent 334, 1267-1272.

11. B. Fulton, C.M. Spencer(1996), Docetaxel, a review of its pharmacodynamic and pharmacokinetics Properties and therapeutic efficacy in the management of metastatic breast cancer, Drugs 51 (6) 1075-1092

12. S.Du and Y.Deng (2006), Studies on the Encapsulation of Oxymatrine into liposomes by Ethanol Injection and pH Gradient Method, Drug development and industrial pharmacy,32: 791-797.

13. P.Crosasso, M. Ceruti, P. Brusa, S. Arpicco, F. Dosio. L. Cattel (2000) Prepartion, characterization and properties of sterically stabilized Paclitaxel- containing Liposomes, J.Control Relaese 63, 19-30.

14. Maria Laura Immordino, Paola Brusa, Silvia Arpicco, Barbara Stella, Franco Dosio, Luigi Cattel(2003) Preapartion, Characterization, Cytotoxicity and pharmacokinetics of liposomes containing docetaxel. Controlled Release 91, 417-429

15. Olbrich, C. and Muller, R.H. (1999), Int. J. Pharm., 180, 31-39.

16. Yong-Zhuo Huang, Jian-Qing Gao, Wen-Quan Liang and Shinsaku Nakagawa (2005). Preparation and Characterization of Liposomes Encapsulating Chitosan Nanoparticles. Biol.Pharma.Bull.28(2) 387-390.

17. Komatsu, H., Kitajima, A. and Okada, S.(1995), Chem.Pharm.Bull., 43, 1412- 1415

18. Levy, M.Y., Schutze, W., Fuhrer, C. and Benita, S. (1994), J. Microencapsulation., 11, 79- 92

19 Takamura, A., Ishii, F., Noro, S., Tanifuji, M. and Nakajima (1984), S., J. Pharm. Sci., 73, 91-94.

20. S. Alamelu and K.Panduranga Rao (1991). Studies on the carboxymethy chitosan- Containing Liposomes for their stability and Controlled release of Dapsone. J. Microencapsulation, vol.8, No, 4, 505-519.

21. Mayer LD, St Onge G(1995).Determination Of free and liposome associated doxorubicin and Vincristine levels in Plasma under equilibrium conditions employing ultra filtration techniques. Anal Biochem. ; 232:149-157.

22. T.Musumeci, C.A. Ventura, I. Giannone, B.Ruozi, L.Montenegro, R.Pignatello, G.Puglisi (2006). PLA/PLGA

nanoparticles for sustained release of docetaxel, International Journal of pharmaceutics 325,172-179.
23.Zur Muhlen, A., Zur Muhlen, E., Niehus, H., and Mehnert, W.(1996), Pharm. Res., 13(9), 1411-1416
24. Siekmann, B. and Westesen, K., Colloids Surf. B, 1994a, 3, 159-175.