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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 16 (6): 295-298, 2013
ISSN 1028-8880 / DOI: 10.3923/pjbs.2013.295.298

Paclitaxel Loaded Niosome Nanoparticle Formulation Prepared via Reverse Phase Evaporation Method: An *in vitro* Evaluation

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Abstract: Niosoms are nanoparticles used in drug delivery systems. Niosomes are prepared by various methods. In this research niosoms were prepared by reverse phase evaporation and the factors affecting the niosomes formation were studied. Percent of paclitaxel pegylated and non-pegylated prepared with Span 60 were 95 and 92, respectively while for those of pegylated and non-pegylated niosomes with Span 20, 94 and 90, respectively. In addition, the average diameters of pegylated and no-pegylated prepared with Span 60 and 20 were determined to be 191, 214, 244 and 284 nm, respectively. The amount of released drug (48 h) from pegylated and non pegylated formulations in the presence of Spans 60 and 20 were 8, 10, 6, 7%, respectively. Cytotoxicities of paclitaxel niosom polyethyleneglycol, paclitaxel niosome and free paclitaxel on MCF-7 cell line after 48 hours were studied by MTT assay. The results showed the formulation prepared with Span 60 is more effective than that of Span 20 and the IC_{50} of the former was decreased twice while IC_{50} of the later decreased 1.5 times.

Key words: Nanoniosome, paclitaxel, polyethyleneglycol, breast cancer

INTRODUCTION

Diagnosis and treatment of cancer have been revolutionized by nanotechnology (Riaz, 1996). Today, reductions of side effects and increase in efficacy of chemotherapeutic agents by employing nanotechnology in medicine are the goal of scientists. Recent advances in nanotechnology have made possible the site specific treatments of animals and human diseases by lowering the drugs' adverse effects (Mozafari, 2006). Carriers injection at nano scale are used with the aim of passing through biological barriers, drug protection and release of optimum dose of drugs (Costantino and Boraschi, 2012). Niosomes are one of these carriers. Niosomes are non ionic surfactant vesicles formed by non-ionic hydration of surfactant or without incorporation of cholesterol and other fats. Their vesicular system can be used as carriers of lipophilic and amphiphilic drugs. Their non ionic nature reduces the toxicity and limits their reaction with cell. This in turn will improve the therapeutic index of drug (Pawar *et al*., 2012).

Niosomes entrap soluble material as do liposomes but the former is more stable at laboratory's atmosphere so they can increase the stability of the drugs. Their production cost is low and their storage is easy (Hofland *et al*., 1992; Malhotra and Jain, 1994). There are different methods to prepare such nanocarriers (Rajera *et al*., 2011). In this study formulations were prepared by reverse phase evaporation employing Spans 60 and 20. Paclitaxel is an effective chemotherapeutic agent which is extracted from the bark of *Taxus brevifolia*. In clinics, paclitaxel is used to treat breast and ovarian cancers (Singla *et al*., 2002). Due to low solubility and limited source of production, paclitaxel is not used alone in clinic. Its solubility in water is less than 3 $\mu g\ mL^{-1}$ (Liggins *et al*., 1997). Therefore, an equal ratio of Cremophore EL, paclitaxel and ethanol was formulated which was used by the addition of normal saline containing 5% dextrose in clinic (Waugh *et al*., 1991). But Cremophore EL poses different side effects including hypersensitivity, neurotoxicity, renal and cardial toxicities (Liebmann *et al*., 1993). Encapsulation of paclitaxel in biodegradable nano or micro carriers (Ruan and Feng, 2003; Mu and Feng, 2003), Liposomes (Sharma *et al*., 1997), core-shell nanoparticles (Oh *et al*., 2005) micelles (Liggins and Burt, 2002) or dendric polymer (Ooya *et al*., 2003) is one of the ways to overcome such obstacle, increase bioavailability and tumor accumulation . In this article the increment in bioavailability of paclitaxel in niosom nanoparticles and high percent of entrapment was achieved.

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MATERIALS AND METHODS

Span 20 and 60, paclitaxel and MTT were purchased from Sigma Chemical Co., USA, polyethyleneglycol 20000 (Kimiagran Co. Iran). Ethanol, Isopropanol (Merk, Germany). RPMI 1640 was obtained from Invitrogen.

Cell Line: MCF-7 cell line was procured from National Cell Bank, Pasteur Institute of Iran, Tehran, Iran.

Preparation of niosome, encapsulation and pegylation of paclitaxel by reverse phase evaporation: Reverse phase evaporation method employed to prepare niosome was modified in this study (34). Niosomes containing paclitaxel were prepared using non ionic surfactants viz., Span 20, Span 60 and cholesterol at defied concentration (Table 1). In brief, cholesterol and surfactant (either Span 20 or Span 60) were dissolved at 30 mL ethanol 98%. Then 40 mg paclitaxel was added and mixed on stirrer at 300 rpm, room temperature for 30 min.

In continuation, solvent phase was evaporated on rotary evaporator. The resultant gel was dissolved in 20 mL normal saline. For pegylated formulation preparation, in addition to the above materials, polyethyleneglycol 20000 was added at the beginning of the reaction.

Sonication and homogenization of nanoparticles: In order to homogenize vesicle, the resultant suspensions were sonicated (Bandelin Sonorex, Germany) at 60 HTz and room temperature for 15 min.

Size determination of nanoparticles: The average hydrodynamic diameter of niosoms were determined by zeta sizer (Zen 3600, Malvern Instrument Ltd., Malvern Worcestershire UK):

$$\text{Encapsulation (\%)} = \frac{\text{Initial paclitaxel concentration (mg)} - \text{paclitaxel concentration in supernatant (mg)}}{\text{Initial paclitaxel concentration (mg)}} \times 100$$

Encapsulation yield: The amount of encapsulated paclitaxel was determined as follows: The suspension containing nanoparticles was centrifuged for 30 min at 13000 rpm and room temperature and the supernatant was collected and the absorbance of each formulation was read at λ 227nm.

The amount of encapsulation was determined as per formula 1. Paclitaxel standard was constructed.

Drug release studies: The amount of released paclitaxel was determined through dynamic membrane diffusion method. Niosomal suspension containing 4 mg paclitaxel was poured into a dialysis bag 9 cut off 12000 Da, Sigma. The dialysis bag containing above suspension was floated in a container on magnetic stirrer at 37°C, 100 rpm containing 20 mL phosphate buffer pH 7.4 which was kept. At the time interval of 3, 5, 7, 9, 21, 24, 27, 30 and 48, samples of 2 mL withdrawn and such amount was replaced with fresh buffer. The absorbencies of the samples were read at 227 nm.

Cellular cytotoxicity: The extent of cytotoxicity was studied on MCF-7 cell line using MTT assay on 96 well plates as reported by Zarei *et al.* (2013). The results were evaluated by Pharm program and IC_{50} of the results for each sample was reported.

RESULTS

Size determination of niosomes: The average hydrodynamic diameter of niosomal nanoparticles pegylated and non pegylated prepared with Spans 60 and 20 were found to be 191, 214, 244 and 284, respectively.

Encapsulation yield: Encapsulation yields of each formulation were determined through constructed paclitaxel standard curve. Percent encapsulation of paclitaxel in niosom nanoparticles pegylated and non pegylated prepared with Spans 60 and 20 were determined from formula 1 to be 92, 95, 90 and 94%, respectively.

***In vitro* studies of paclitaxel release:** It was found that apart from kind of surfactants used to prepare the formulations, the amount of released paclitaxel was low. But the role played by pegylation was prominent in other words, prepared pegylated formulations showed lower paclitaxel release as compared to non-pegylated formulation. Also, such differences in niosomal preparation with Span 20 were significant (Fig. 1, 2).

Evaluation of cellular cytotoxicity effect: It was initially found that the higher concentration of nanoparticles devoid of drugs did not affect the cell line, thereby, it was

Table 1: Ratios of surfactants, paclitaxel and cholesterol to prepare niosomes

PEG 2000 (mg)	Cholesterol (mg)	Surfactant (mg)	Drug (mg)	Drug/surfactant/cholesterol ratio	Surfactant
20	20	100	40	2:5:1	Span 20
-	20	100	40	2:5:1	Span 20
20	20	100	40	2:5:1	Span 60
-	20	100	40	2:5:1	Span 60

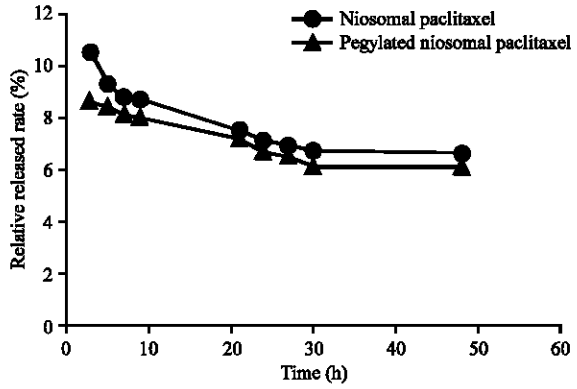

Fig. 1: Paclitaxel release from two niosomes pegylated and non-pegylated using Span 60

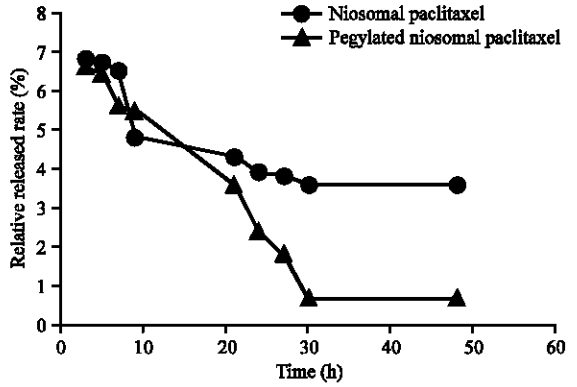

Fig. 2: Paclitaxel release from two niosomes pegylated and non-pegylated using Span 20

considered to be safe. It was also observed that toxicity of paclitaxel in formulations; niosome-polyethyleneglycol and niosome-non polyethyleneglycol prepared with Spans 60 and 20 was higher than toxicity of free paclitaxel. However, this increment in toxicity was higher in formulation prepared with Span 60 in comparison to Span 20. Furthermore, determined IC_{50s} for pegylated and non pegylated preparations with Span 60 and 20 were 24, 48, 35 and 57 μm, respectively while the IC_{50} for free drug was determined to be 96 μM. The results showed that the role of pegylation on imposed toxicity.

DISCUSSION

It is found that about 40%of compounds prepared by combinatorial chemistry are compounds with low solubility which is considered a challenge in formulating them. Using drug delivery systems is an approach for improving solubility and maintaining physiological activity of such drugs. In this study, paclitaxel was chosen as a model due to its low solubility in order to encapsulate in niosome (Sezgin-Bayindir and Yuksel, 2012). Non ionic surfactants were selected because of their low toxicity. Cholesterol was added to the formulation as a membrane stabilizer. Although, interaction between cholesterol and bilayered surfactant depends upon structure of lipid and its lipophilic head but incorporation of cholesterol causes the loss in transition state of gel to lipid bilayer (Paolino *et al*., 2008). Unlike most of other related studies, employ the equal ratios of cholesterol and surfactant (Paolino *et al*., 2008; Bayindir and Yuksel, 2010), we used the ratio of 1(cholesterol): 5 (surfactant) where nanoparticles of appropriate properties were obtained. The method used in this study to prepare niosomes is easy, appropriate yield and low cost benefit. This study also showed paclitaxel can highly be loaded onto niosoms. It causes increase in bioavailability of drug. The niosomes prepared by this method, in addition to keeping paclitaxel's anticancer properties, its efficiency was also increased. The toxicity of paclitaxel in formulated forms was higher than toxicity of free drug. This could be attributed to slow release of drug from the nanoparticles. It is reported that by increasing the amount of surfactant's HLB, the size of nanoparticles would increase (Yoshioka *et al*., 1994) which was observed by us in this study. Yield of encapsulation was higher in niosomes prepared in presence of Span 60 which in contradiction with results obtained by (Bayindir and Yuksel, 2010). This could be related to the method of nanoparticles preparation. Stability of prepared formulations in lyophilized form was studied at the end of first and third months of storage. The amount of drug released was negligible. Effects of pegylation on formulation were studied. These result tallies with the result obtained by Cosco *et al*. (2009) indicating such molecule increases the drug load. Unlike studies carried out by Mukherjee *et al*. (2007), presence of polyethyleneglycol in formulation caused decrease in size of nanoparticles. Apart from surfactants, pegylation caused toxicity increment and slow drug release. Finally, the obtained results suggest niosomal nanoparticles can be used as an appropriate nanocarrier of paclitaxel and such formulation can be further *in vivo* studied.

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