PREPARATION AND PHYSICOCHEMICAL EVALUATION OF TOCOPHERYL POLYETHYLENE GLYCOL SUCCINCT 1000, 5000 (TPGS $_{1K, 5K}$) MICELLES FOR DELIVERY OF PACLITAXEL

[1]MAHBOUBEH REZAZADEH, [2]JABER EMAMI, [3]FARSHID HASSANZADEH

[1]Department of Pharmaceutics and Novel Drug Delivery Systems Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R. Iran
[2]Department of Pharmaceutics, School of Pharmacy and Pharmaceutical Sciences and Isfahan Pharmaceutical Research Center, Isfahan University of Medical Sciences, Isfahan, I.R. Iran
[3]Department of Medicinal Chemistry, School of Pharmacy and Pharmaceutical Sciences and Isfahan Pharmaceutical Research Center, Isfahan University of Medical Sciences, Isfahan, I.R. Iran,
E-mail: [1]rezazade@pharm.mui.ac.ir, [2]emami@pharm.mui.ac.ir, [3]hasanzadeh@pharm.mui.ac.ir

Abstract- The objective of the present study was to evaluate the potential of paclitaxel (PTX) loaded mixed micelles fabricated from tocopheryl polyethylene glycol succinate 1000, 5000 (TPGS $_{1K, 5K}$) as a sustained release system. Here, we developed a micellar system with a newly synthesized $TPGS_{5k}$ polymer, which showed lower CMC of 0.078 mg/ml compared with traditional micelles prepared with $TPGS_{1k}$ to achieve sustained and controlled drug delivery. The blank and drug loaded micelles were prepared in different weight ratios of $TPGS_{1K}$/$TPGS_{5K}$ by solvent evaporation technique. Physicochemical properties of the micelles such as critical micelle concentration, micelle size, entrapment efficiency, and release properties were investigated in detail. The results showed that mixed micelles made of TPGS $_{1K}$ and TPGS $_{5K}$ (weight ratio 3:7) had lowest CMC value, indicating that they were most stable towards dilution among micelles. Particle sizes and zeta potentials of the micelles were in the range of 50.3–102 nm and 18.5–28.3 mV, respectively. The drug entrapment efficiencies of the micelles were within 63.6–92.5% (w/w). The results revealed that adding TPGS 5K could increase the drug loading. The drug loaded micelles exhibited a sustained release behavior in dissolution medium. From the micellar formulation, approximately 30% of PTX was released within 10 h at 37◦C, afterwards, the release rate slowed down and was almost complete after 5 days. In comparisons, 50% of PTX was released rapidly from Anzatax® (PTX in Cremophor El) within 5 h and it was released completely after 30 h.

Index Terms- Paclitaxel, α-tocopheryl polyethylene glycol succinate, Micelles

I. INTRODUCTION

Paclitaxel (PTX), a microtubule stabilizing anti-proliferative
Agent, is broadly used in treatment of aggressive human cancers, especially in carcinomas of the lungs, breast and ovary [1]. Because of the low water solubility, PTX is commercially available in a solution composed of a 50:50 (v/v) mixture of Cremophor® EL (polyethoxylated castor oil) and dehydrated ethanol marketed as Taxol®. Unfortunately, Cremophor® EL induces some serious adverse reactions such as hypersensitivity, nephrotoxicity and neurotoxicity after intravenous (i.v.) administration [1], [2].additionally, Taxol® has short-term physical stability upon dilution due to the precipitation of PTX in the aqueous media. Hence, many attempts have been conducted to find alternative carriers for PTX that can overcome poor aqueous solubility of PTX, enable delivering PTX more specifically to the target tissue, and produce fewer adverse effect on normal cells. Numerous drug delivery systems such as parenteral emulsions [3], lipid and polymeric nanoparticles [4], [5], liposome [6], water soluble prodrugs [7] and polymeric micelles [8], [9], [10] have been evaluated for delivery of PTX into the body and targeted cancer cells.

In particular, polymeric micelles, self-assemblies of amphiphilic block or grafted copolymers, have been extensively studies. These copolymers form a core-shell structure in aqueous media, providing a hydrophobic space in the core where hydrophobic drugs can be solubilized. α-tocopheryl polyethylene glycol succinate 1000 (Vitamin E TPGS or$TPGS_{1k}$) is a water-soluble derivative of natural vitamin E. Vitamin E conjugated with polyethylene glycol 1000(PEG) has amphiphilic structure with lipophilic alkyl tail and hydrophilic polar head portion. Its bulky structure and large surface area makes it an excellent emulsifier, solubilizer, and bioavailability enhancer of hydrophobic drugs [11]. It has also been found that vitamin E TPGS could inhibit P-glycoprotein (P-gp) mediated multidrug resistance and thus greatly enhance the cytotoxic effect of anticancer agents substrating P-gp such as paclitaxel (PTX) [11], [12]. In addition, vitamin E analogues such as TPGS, has attracted attention as a unique anti-cancer drug for its ability to induce DNA synthesis arrest and apoptosis in various cancer cells. Thus, using TPGS as carrier can promote synergistic cytotoxic effect with encapsulated anti-cancer agents. Nevertheless, one of the disadvantages of $TPGS_{1K}$ in micelle formulation is its high CMC (0.2mg/ml), which decreases the stability of micelles in physiological environment

[11]. Also, the chain length of PEG in TPGS is not long enough to ensure the micelles to extend blood circulation times to reduce liver accumulation. Thus, till now, TPGS was usually used together with other lipids or synthetic copolymers to form micelles. Recently we synthesized a new $TPGS_{5k}$ amphiphilic polymer via reaction between tocopheryl succinate and methoxy polyethylene glycol of molecular weight 5000 (mPEG5000). This new synthesized $TPGS_{5k}$ showed much lower CMC value compared with the traditional $TPGS_{1K}$. This change makes it possible to form stable micelles by combination of TPGS $_{1k}$ without any other polymers or lipids. In fact, long chain PEG helps the micelles escape from being recognized and eliminated by the reticuloendothelial system (RES). The current work focuses on preparation of mixed micelles with different weight ratios of TPGS $_{5K}$ and TPGS $_{1K}$. PTX was loaded to the micelles as model drug. Micellar size, zeta potential, the drugs entrapment efficiency (EE) and release behavior of the drug from the micelles were investigated in detail.

II. MATERIALS AND METHODS

Materials

α-tocopherol succinate, α-Tocopheryl polyethylene glycol 1000 succinate (TPGS 1K), and paclitaxel (PTX) purchased from Sigma Chemical Co. (St. Louis, MO). α-tocopherol succinate, α-Tocopheryl polyethylene glycol 5000 succinate (TPGS 5K) derivative was synthesized in our laboratory. HPLC grade acetonitrile and methanol were procured from Caledon (Ontario,Canada). PTX in Cremophor® EL (Anzatax®, 30 mg/5ml) was provided by Hospira (Mulgrave, Australia).

Micelle preparation

To prepare empty mixed micelles, TPGS 1K and TPGS 5K in different weight ratios (0:10 to 10:0) were dissolved in chloroform and the organic solvent was subsequently removed by rotary vacuum evaporation. The film formed was hydrated with a suitable amount of phosphate buffered saline (FBS). The resulting mixture was filtered through a 0.2 μm Nylon filter. To obtain drug-loaded micelles, PTX dissolved in methanol and added to TPGS solutions in chloroform. The micelle preparation steps described above were repeated [11].

Determination of critical micelle concentration

The critical micelle concentrations (CMC) of mixed micelles were determined by fluorescence measurement using pyrene as a hydrophobic fluorescence probe. A 1.2 mg/ml solutions of TPGS $_{1k,}$ $_{5k}$ in different weight ratios were prepared in dichloromethane. Different volume of this solution was added to 15 ml empty vials. Then 50 μl of 1.8×10^{-4} M solution of pyrene in dichloromethane was added in every vial and mixed well. The dichloromethane was left to evaporate for 24 h in order to form a pyrene film in the vial. Finally,15 ml of distilled water was added to the vials to get a final pyrene concentration of 6.0×10^{-7} M for each vial and the micelle solutions with concentrations ranging from 10^{-4} mg/ml to 1 mg/ml. The solutions were kept on a shaker at 37 C for 24 h to reach equilibrium before fluorescence measurement. The fluorescence emission spectra of pyrene were recorded using spectrofluorometer (Jasco FP 750, Tokyo, Japan) with the excitation wavelength set at336 nm. From the pyrene emission spectra, the intensity ratio of the first peak to the third peak was plotted against the logarithm of polymer concentration. Two tangents were then drawn and the CMC values were taken from the intersection between the two tangents [8].

Particle size and zeta potential of micelles

The average hydrodynamic diameters and zeta potentials of the micelle solutions (1 mg/ml) in phosphate buffer saline (PBS, pH 7.4) were measured by dynamic light scat-tering (DLS) using a ZetaSizer (3000HS, Malvern Instruments Ltd.,Malvern, UK).

Determination of the drug loading level

To estimate the drug loading content, 2 mg of the lyophilized sample was dissolved in 50 ml of acetonitrile and the solution was ultrasonicated for 10 min to dissolve PTX-loaded micelles entirely. The resultant solution was filtered through 0.45 μm filter unit and PTX concentration was determined by HPLC method developed and validated in our laboratory. Chromatographic separation was per-formed using a reverse-phase C18-Bondapak (3.9 mm × 250 mm) column. The mobile phase consisted of potassium dihydrogenphosphate (0.01 M)/acetonitrile (35:65) with final pH adjusted to3.5 ± 0.1 with ortho-phosphoric acid. The mobile phase eluted at the flow rate of 1.5 ml/min and the effluent was monitored at227 nm using UV detector. Column temperature was kept at 40◦Cand 30 μl of the sample was injected into the HPLC column. The calibration curve was constructed in the range of 0.5– 20μg/ml (r2> 0.998), the inter- and intra-day precision and accuracy were less than 12.87%.The drug entrapment efficiency (EE) and drug loading (DL) were calculated by the following equations:

EE%=(weight of the drug in the micelle)/(weight of the feeding drug)×100

DL%=(weight of the drug in the micelle)/(weight of the micelles)×100

In vitro drug release studies

2 mg of the lyophilized powder of PTX-loaded micelles was dissolved in 2 ml of phosphate buffer solution (0.1 M, pH: 7.4). The resulting solution was placed in dialysis bag (MWCO: 10 kDa), and the bag was immersed in a glass beaker with 200 ml phosphate buffer (0.1 M, pH: 7.4) containing 0.5%tween 80 to provide sink condition with agitation of 100 rpm at37◦C. The samples were taken from the release medium at the pre-determined time intervals and replaced with fresh buffer [9]. The content of PTX was determined by the above described HPLC method.

II. RESULTS AND DISCUSSION

The CMC of different micelles in PBS (pH: 7.4) are listed in Table 1. The results showed that mixed micelles made of TPGS $_{1K}$ and TPGS $_{5K}$ (weight ratio 3:7) had lowest CMC value, indicating that they were most stable towards dilution among micelles.

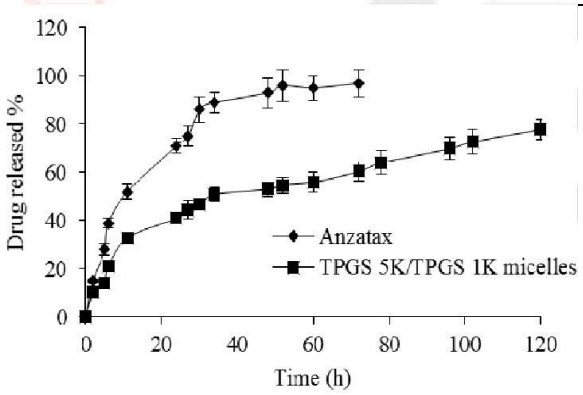
Table 1: CMC values of TPGS $_{5K}$/TPGS $_{1K}$ micelles

	[Formulations (TPGS 5K: TPGS 1K) (weight ratios)]		
	10:0	7:3	5:5
CMC (µg/ml)	65.2	78.2	100

The size of PTX-loaded micelles was larger than those of the blank micelles. This size increase may be due to the drug loading in the micellar core. The zeta potentials were also reduced after the drug was loaded due to the change of size and surface charge density of the micelles (Table 2). The amount of PTX in the micellar core was determined by the reverse phase-HPLC. The results revealed that adding TPGS $_{5K}$ could increase the drug loading. The drug loading of mixed micelles made of TPGS 1K/TPGS 5K (3:7 weight ratio) was about 28%.

Table 2: Drug loading of PTX in micelles

composition	$TPGS_{5K}$	$TPGS_{5K}$:$TPGS_{1K}$ (7:3)	$TPGS_{5K}$: $TPGS_{1K}$ (5:5)
Drug loading (%)	31 ±2.6	28.4 ±1.9	19.8±2.8



Fg.1. Release profile of PTX from Anzatax® and $TPGS_{5K}$/TPGS $_{1K}$ mixed micelles

In vitro PTX release profile from TPGS 1K/TPGS 5K micelles (weight ratio3:7) is shown in Fig. 1. From the micellar formulation, approximately 30% of PTX was released within 12 h at 37◦C, afterwards, the release rate slowed down and was almost complete after 5 days.

In comparisons, 50% of PTX was released rapidly from Anzatax® within 5 h and it was released completely after 30 h. The sustained drug-release from the micelles might be due to the relatively high lipophilicity of PTX and also strong hydrophobic interactions between PTX and the core of micelles. In the majority of earlier published reports, the release rate of PTX from polymeric micelles, especially in initial stages, was much faster than that of our developed micelles. An enhanced sustained release of PTX observed in the current study could be explained by the strong hydrophobic interactions between PTX and the bulk lipophilic portion of TS molecules formed the hydrophobic core of the micelles.

CONCLUSION

The newly TPGS $_{5K}$ was successfully synthesized in our laboratory. The stable micelles with small sizes and low CMC value were prepared by combination of TPGS $_{5K}$ and TPGS $_{1K}$, which presented an excellent candidate for delivery of PTX to the cancer cells.

REFERENCES

[1] J Emami, M, Rezazadeh, J. Varshosaz, M. Tabbakhian, A. Aslani. "Formulation of LDL Targeted Nanostructured Lipid Carriers Loaded with Paclitaxel: A Detailed Study of Preparation, Freeze Drying Condition, and In Vitro Cytotoxicity', 20.12, doi:10.1155/2012/358782

[2] SC Kim, DW Kim, YH Shim. "In vivo evaluation of polymeric micellar paclitaxel formulation: toxicity and efficacy" J Control Release 72:191–202. 2001

[3] He L, Wang G, Zhang Q. An alternative paclitaxel microemulsion formulatiom: hypersensitivity evaluation and pharmacokinetic profiles. Int J Pharm 250:45–50. 2003

[4] E, Bernabeua, G, Helguera, M, Legaspia, L, Gonzalez. Paclitaxel loaded PCL–TPGS nanoparticles: in vitro and in vivo

performance compared with Abraxane®. Colloids Surf B Biointerfaces 113:43–5. 2014

[5] MJ Hawkins, P Soon-Shiong, N Desai. Protein nanoparticles as drug carriers in clinical medicine. Adv Drug Deliv Rev 2008:60:876–85.

[6] GJ Fetterly, RM Straubinger. Pharmacokinetics of paclitaxel containing liposomes in rats. AAPS Pharm Sci 5:123–9.2003

[7] B. Elsadek, R. Graeser, N. Esser et al., "Development of a novel prodrug of paclitaxel that is cleaved by prostate-specific antigen: an in vitro and in vivo evaluation study," European Journal of Cancer, vol. 46, no. 18, pp. 3434–3444, 2010.

[8] J Emami, M, Rezazadeh, F, Hasanzadeh, H, Sadeghi, Mostafavi, M, Minaiyan, M Rostami, Neal Davies" Development and in vitro/in vivo evaluation of a novel targeted polymeric micelle for delivery of paclitaxel" Int. J. Biological Macromolecules 80, 29–40, 2015

[9] M, Rezazadeh, J, Emami, F, Hasanzadeh, H, Sadeghi, M, Minaiyan, A, Mostafavi, M, Rostami, A, Lavasanifar. In vivo pharmacokinetics, biodistribution and anti-tumor effect of paclitaxel-loaded targeted chitosan-based polymeric micelle. Drug Deliv, 23: 1707–1717:2016

[10] G. Qu, Z. Yao, C. Zhang, X. Wu, Q. Ping, PEG conjugated N-octyl-O-sulfatechitosan micelles for delivery of paclitaxel: in vitro characterization andin vivo evaluation, Eur. J. Pharm. Sci. 37: 98–105. 2009

[11] Y, Mi, Y Liu, S Feng. Formulation of Docetaxel by folic acid-conjugated D-a-tocopheryl polyethylene glycol succinate 2000 (Vitamin E TPGS2k) micelles for targeted and synergistic chemotherapy, Biomaterials 32: 4058-4066: 2011

[12] H, Zhu , H, Chen, X, Zeng, Zh, Wang, X, Zhang, Y, Wu, Y, Gao, J, Zhang, K, Liu, R, Liu, L, Cai, L, Mei, S, Feng. Co-delivery of chemotherapeutic drugs with vitamin E TPGS by porous PLGA nanoparticles for enhanced chemotherapy against multi-drug resistance Biomaterials 35, 2391-2400, 2014

★★★