Liposomes With High Encapsulation Capacity for Paclitaxel: Preparation, Characterisation and *In Vivo* Anticancer Effect

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ABSTRACT: Paclitaxel (PTX) is approved for the treatment of ovarian and breast cancer. The commercially available preparation of PTX, Cremophor EL® is associated with hypersensitivity reactions in spite of a suitable premedication. In general, the developed liposomal PTX formulations are troubled with low PTX encapsulation capacity (maximal content, 3 mol%) and accompanied by PTX crystallisation. The application of "pocket-forming" lipids significantly increased the encapsulation capacity of PTX in the liposomes up to 10 mol%. Stable lyophilised preparation of PTX (7 mol%) encapsulated in the liposomes composed of SOPC/POPG/MOPC (molar ratio, 60:20:20) doped with 5 mol% vitamin E had the size distribution of 180–190 nm (PDI, 0.1) with ζ-potential of −31 mV. Sucrose was found to be a suitable cryoprotectant at the lipid:sugar molar ratios of 1:5–1:10. This liposomal formulation did not show any evidence of toxicity in C57BL/6 mice treated with the highest doses of PTX (100 mg/kg administered as a single dose and 150 mg/kg as a cumulative dose applied in three equivalent doses in 48-h intervals). A dose-dependent anticancer effect was found in both hollow fibre implants and syngenic B16F10 melanoma mouse tumour models. © 2009 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 99:2309–2319, 2010

Keywords: paclitaxel; liposomes; extrusion; particle size; lyophilisation; stability; melanoma; B16F10; hollow fibre implants; nanotechnology

INTRODUCTION

PTX is one of the most important compounds that emerge from a natural source. This drug is approved for the treatment of ovarian and breast cancer and is one of the most exciting anticancer molecules currently available.[1,2] However, a suitable drug formulation still remains a problem, because PTX has a low therapeutic index owing to its high lipophilic character and correspondingly low solubility in water. The commercially available injectable preparation Taxol® is a sterile solution of PTX in Cremophor EL® (polyethoxylated castor oil) and dehydrated alcohol. Present day cancer chemotherapy with PTX is associated with hypersensitivity reactions in spite of a suitable premedication with corticosteroids and anti-histamines.[3] Hence, the development of an improved delivery system for PTX is of high importance. Current approaches to the improvement are focused mainly on the development of formulations that are devoid of Cremophor EL®, investigation of the possibility of a large-scale preparation and questing for a longer-term stability. These different approaches have shown some promising possibilities to replace Taxol® by a less irritable preparation such as: (a) micelle formulations,[4] (b) water-soluble prodrug preparations,[5] (c) enzyme-activatable prodrug preparations conjugated with antibodies or albumin,[6,7] (d) parenteral emulsions,[8] (e) microspheres,[9] (f) cyclodextrins[10] and (g) nanocrystals.[11] However, none of these alternatives has reached the stage of replacing Taxol® in the clinic yet. Nonliposomal formulations of PTX based on protein-bound particles (Abraxane®) were recently approved by FDA. The present-day situation in PTX drug formulations is well reviewed by Hennenfent and Govindan.[12]

Additional Supporting Information may be found in the online version of this article.

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Abbreviations: EE_{PTX}, encapsulation efficiency of paclitaxel; DLS, dynamic light scattering; MOPC, 1-oleoyl-2-hydroxy-*sn*-glycero-3-phosphatidylcholine; MOPG, 1-oleoyl-2-hydroxy-*sn*-glycero-3-phosphatidylglycerol; MTD, maximum tolerated dose; PEG-POPE, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[poly(ethyleneglycol)^{2000}]; PDI, polydispersity index; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-phosphatidylglycerol; PTX, paclitaxel; SOPC, 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine.

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Liposomes, lipid membrane vesicles, represent potentially versatile drug carriers for a wide range of drugs.[13] Recent advances in this area have led to the development of some products for human medicine, for example, amphotericin and liposomal doxorubicin,[14] and many other liposomal formulations of drugs are in various stages of clinical trial. Various liposome-based PTX formulations were developed in order to improve the drug therapeutic efficiency and to eliminate its negative side-effects. Within the range of the liposomal PTX preparates, different approaches had been designed, which has resulted in the preparation of conventional liposomes,[15,16] sterically stabilised liposomes,[17,18] immunoliposomes,[19,20] cationic liposomes,[21,22] and magnetoliposomes.[23] The liposomal PTX formulations have been successfully tested on various experimental *in vivo* models. In comparison with PTX in Cremophor EL®, the liposomal PTX was shown to exhibit a lower toxicity, higher efficiency and an increased MTD. However, the maximal achieved encapsulation capacity of the conventional liposomal formulations for PTX was only about 3 mol%. There have been reports to suggest that the PTX encapsulation can be further increased using lysophospholipids in the liposome formulation. These lipids increase the membrane bilayer fluidity and create bilayer "pockets," in which the hydrophobic molecules such as PTX can be encapsulated.[24] Yet, no studies have been reported on PTX encapsulated in pocket-forming liposomes as regards their physical-chemical stability as well as biological activity. In this study we investigated the usableness of pocket-forming lipids for the preparation of stable and nontoxic PTX liposomes. This basic liposomal carrier could be used for the development and construction of next generation of advanced targeted PTX delivery system. We report improvements in this approach leading to a new optimal liposomal PTX formulation (7 mol% PTX) that is well tolerated and efficient for the treatment of experimental cancer in mouse models. Moreover, this formulation is stable in the lyophilised form during storage.

MATERIALS AND METHODS

Chemicals

Lipids comprising SOPC, MOPC, POPG, PEG-POPE and MOPG were purchased from (Avanti Polar Lipids Alabaster, AL). Vitamin E was obtained from Sigma–Aldrich (Prague, Czech Republic). PTX (purity of 97%, HPLC) was purchased from Houser Chemical Research Inc. (Boulder, CO). All the organic solvents used (reagent or HPLC grade) were from Sigma–Aldrich (Czech Republic).

Methods for the Preparation of PTX Liposomal Formulations

The preparation of the liposomes by lyophilisation from 2-methyl-butan-2-ol was done as described in literature.[15] Briefly, lipids, vitamin E and PTX were solubilised in 2-methyl-butan-2-ol (Sigma–Aldrich, Czech Republic). The mixture of lipids and PTX (12 mg/mL; the total volume of 1 mL) was frozen at −80°C and lyophilised for 24 h in a Lyovac GT2 instrument (Finaqua, Finland). Stepwise hydration of the lipids in PBS was accomplished via step-by-step addition of an aqueous phase to the lyophilisate (20-μL additions during 20 min, total volume of 200 μL) under continual magnetic stirring. After lipid hydration, the volume was adjusted to 1 mL in total and optional extrusion through 0.2-μm polycarbonate filters was performed.

Liposomes containing vitamin E and PTX were prepared by the proliposome-liposome method and by hydration of a lipid film followed by extrusion through 0.2-μm polycarbonate filters in an analogous way to that described previously by Turánek et al.[25,26] The hand operated device Mini-Extruder (Avanti Polar Lipids Alabaster, AL) was used for the extrusion of small volumes of liposomes (up to 1 mL). Large volumes of the liposomes were extruded by means of a high pressure cell (Millipore Billerica, MA) linked with FPLC instrument (Pharmacia, Uppsala, Sweden).[26] The liposomes were prepared of SOPC, POPG and MOPC according to the general composition formula: molar ratio SOPC/POPG/MOPC, $80 - X{:}X{:}20$; where X is 0, 10, 20 or 30 mol% (cf. Tab. 1). The content of PTX was 7 mol%. The freezing and thawing step was omitted in order to prepare oligolamellar liposomes with low water content.

Table 1. Lipids Used for the Preparation of Conventional and Sterically Stabilised Liposomal PTX Formulations

Preparate Formulation	Lipid Composition (Molar Ratio)		
Conventional Liposomes	SOPC	POPG	MOPC
1	80	0	20
2	70	10	20
3	60	20	20
4	50	30	20
Preparate Formulation	**Lipid Composition (Molar Ratio)**		
Sterically Stabilised Liposomes	SOPC	PEG-POPE	MOPC
5	75	5	20

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