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Research paper

Polymeric Micelles for parenteral delivery of Sagopilone: Physicochemical characterization, novel formulation approaches and their toxicity assessment *in vitro* as well as *in vivo*

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ABSTRACT

Purpose: The block copolymers PEG_{2000}-*b*-PLA_{2200}, PEG_{2000}-*b*-PCL_{2600} and PEG_{5000}-*b*-PCL_{5000} have been currently identified as optimal solubilizing agents for Sagopilone, a poorly water-soluble anticancer drug. In the present study, the stability, formulation feasibility and *in vitro* as well as *in vivo* toxicity were evaluated.

Methods: Dispersion media, storage conditions, and dilutions were varied for stability assessment. The critical micelle concentration (CMC) was determined using a fluorescent probe technique. Lyophilizates and polymeric films were investigated as formulation options. Furthermore, the toxicity was studied *in vitro* and *in vivo* using HeLa/MaTu cells and a nude mouse model, respectively.

Results: A drug–polymer ratio as low as 1:20 (w/w) was sufficient to solubilize Sagopilone effectively and to obtain stable dispersions (24 h: drug content $\geqslant$95%). Although the micelles exhibited a similar thermodynamic stability (CMC: 10^{-7}–10^{-6} M), PEG-*b*-PCL micelles were kinetically more stable than PEG_{2000}-*b*-PLA_{2200} (24 h at 37 °C: drug content $\geqslant$90% compared to 30%, respectively). Lyophilization of PEG-*b*-PCL micelles and storage stability of solid drug-loaded PEG_{2000}-*b*-PLA_{2200} films (3 m, 6 °C: drug content of (95.6 ± 1.4)%) were demonstrated for the first time. The high antiproliferative activity has been maintained *in vitro* (IC_{50}<1 nM). Carrier-associated side effects have not been observed *in vivo* and the maximum tolerated dose of micellar Sagopilone was determined to be 6 mg/kg.

Conclusion: The results of this study indicate that polymeric micelles, especially PEG-*b*-PCL micelles, offer excellent potential for further preclinical and clinical cancer studies using Sagopilone.

1. Introduction

Solubilization represents one of the major challenges in formulation development nowadays since approximately 40% of the new compounds in drug discovery are poorly water-soluble [1]. This is of particular concern in the parenteral delivery field because the number of approved excipients is limited. Furthermore, currently used solubilizers such as Cremophor®EL have been implicated in clinically important adverse effects and unfavourable alterations of the pharmacokinetics of drugs as shown for paclitaxel [2].

Sagopilone (Fig. 1) is a novel, poorly water-soluble anticancer drug belonging to the group of epothilones that is administered parenterally [3,4]. The epothilones present a novel class of microtubule-stabilizing anticancer drugs originally occurring in *Sorangium cellulosum*. Their mechanism of action is similar to paclitaxel, but they exhibit superior features relative to the latter. Besides their activity against various tumour types, they show low susceptibility to key tumour resistance mechanisms *in vitro*, and most importantly, *in vivo* [5]. Thus, they are effective in tumours resistant to paclitaxel making them very likely to become successors to taxane therapy. Sagopilone (Fig. 1) is a synthetic epothilone derivative, which is currently under clinical trial evaluation [6]. Dosing of Sagopilone is limited due to the occurrence of peripheral neuropathy. This is a typical side effect of epothilones, which recently gave reason to the refusal of the marketing authorisation for the epothilone derivative Ixabepilone by the European Medicines Agency (EMEA) [7]. The agency concluded that the benefits in the treatment of breast cancer with Ixabepilone did not outweigh its risks due to neuropathy.

Thus, an optimal delivery system for this class of anticancer drugs requires (a) solubilization of the drug, (b) accumulation of

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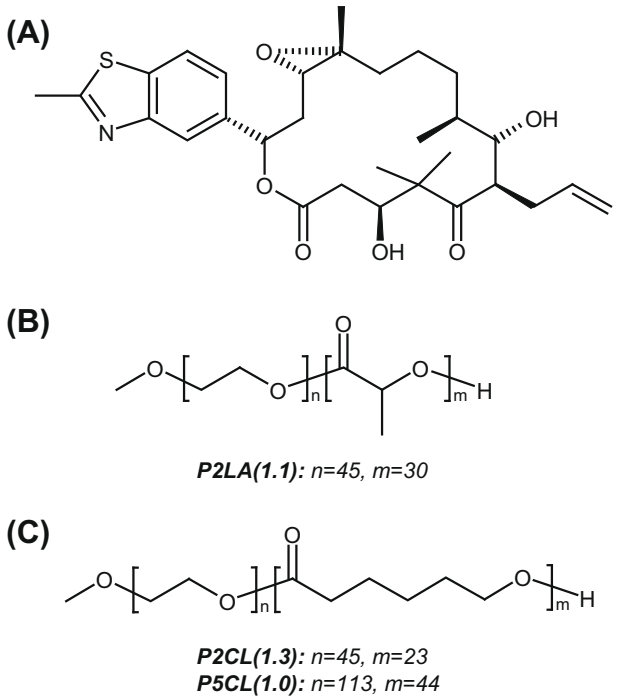

Fig. 1. Structural formula of (A) Sagopilone, (B) PEG-*b*-PLA and (C) PEG-*b*-PCL.

the drug at the tumour site due to enhanced permeation and retention (EPR-effect) [8,9], and (c) reduction of drug-related adverse effects at non-tumour sites. Among several approaches, polymeric micelles offer great potential to meet these demands [10–13] with regard to solubilization [1,14–16], vehicle safety after administration [17,18] and passive tumour targeting [19,20].

Until now, numerous publications have described various polymeric micellar systems with respect to solubilization and *in vivo* performance using different drugs and various animal models. For this reason, the results are difficult to compare. In our previous study, amphiphilic block copolymers composed of poly(ethylene glycol) (PEG) and a biodegradable polyester block of poly(lactide) (PEG-*b*-PLA) or poly(ε-caprolactone) (PEG-*b*-PCL) were investigated with regard to the solubilization of Sagopilone for parenteral delivery [21]. As a result, three polymers along with the appropriate method of preparation were selected as optimal solubilizing agents. The polymers used were: PEG_{2000}-*b*-PLA_{2200}, PEG_{2000}-*b*-PCL_{2600} and PEG_{5000}-*b*-PCL_{5000} (Fig. 1) abbreviated as P2LA(1.1), P2CL(1.3) and P5CL(1.0), respectively, in which the number in parentheses details the hydrophobic/hydrophilic ratio (w/w) of the block copolymer.

A critical point for formulation development is the stability of polymeric micelles [22]. They have to be stable both prior to clinical application and after intravenous (i.v.) administration, since intact micelles are considered an important prerequisite for passive tumour targeting. The stability of polymeric micelles is often considered sufficient in general due to their low critical micelle concentration (CMC) values. However, this view disregards the kinetic stability, which may exhibit serious differences depending on the nature and state of the micellar core [1], especially important in the field of drug delivery. Thus, the selection of a core-forming block providing a high degree of kinetic stability in conjunction with a slow rate of disassembly is described as a strategy for the preparation of micelles that stay intact until reaching the tumour site [23] besides other approaches such as core-crosslinking [24] or the chemical modification of the core-forming block [25,26]. Examining a set of PEG-*b*-PCL polymers, Liu et al. showed superior *in vitro* as well as *in vivo* stability of P5CL(1.0) [23]. A significant portion of the copolymer remained assembled as intact micelles even 24 h after administration of thermodynamically unstable micelles (2 mg/kg body weight) that would likely fall to concentrations below the CMC following distribution [23]. In the present work, a comparative study of the physicochemical stability of PCL- and PLA-containing micelles was performed, assuming that PCL-containing cores exhibit a higher stability due to their nature (higher hydrophobicity) and state (semi-crystalline) compared to amorphous poly(D,L-lactide).

In addition, the applicability of polymeric micelles to clinical development requires stable formulations with sufficient shelf-life. Since the polymers used are sensitive to hydrolytic degradation, aqueous dispersions of the micelles are not suitable for ready-to-use formulations. This issue has been rarely addressed, especially for PEG-*b*-PCL micelles. With regard to the semi-crystalline nature of PCL, potential aggregation has to be taken into account during freeze-drying. With this in mind, the feasibility of lyophilization was studied using different conditions to prevent crystallization of PCL and provide a storable formulation of PEG-*b*-PCL micelles. As an alternative to lyophilization, solid drug-loaded polymeric films of PEG-*b*-PLA were investigated as a novel approach for stabilizing parenteral formulations.

Following the physicochemical and formulation studies, the *in vitro* as well as the preclinical *in vivo* toxicity has been studied to determine the safety profile of the carriers and the maximum tolerated dose (MTD) of the drug-loaded micelles for future *in vivo* tumour efficacy studies.

2. Materials and methods

2.1. Materials

Sagopilone was obtained from Bayer Schering Pharma AG (Berlin, Germany). The block copolymers poly(ethylene glycol)-*b*-poly(ε-caprolactone), namely PEG_{2000}-*b*-PCL_{2600} and PEG_{5000}-*b*-PCL_{5000} (abbr.: P2CL(1.3) and P5CL(1.0), respectively), and the poly(ethylene glycol)-*b*-poly(D,L-lactide) PEG_{2000}-*b*-PLA_{2200} (abbr.: P2LA(1.1)) were purchased from Polymer Source Inc. (Dorval, Canada). Pyrene, sucrose, trehalose and mannitol were obtained from Merck KGaA (Darmstadt, Germany). Hydroxypropyl-β-cyclodextrin (abbr.: HPβCD) was purchased from Roquette (Lestrem, Fance). Polyvinylpyrrolidone (abbr.: PVP, Kollidon® 17PF, M_r = 7000–11,000 g/mol) was purchased from BASF (Ludwigshafen, Germany). All other ingredients were obtained in analytical quality.

2.2. Micelle preparation and drug loading

Loading of Sagopilone within block copolymer micelles was done by the appropriate method of preparation as described previously [21]. In brief, sonication was used to prepare PEG-*b*-PCL micelles by simply weighing the polymer and Sagopilone, adding phosphate buffer (0.05 M, pH 7.4) and sonication for 10 min. Micelles composed of the PEG-*b*-PLA polymer P2LA(1.1) were prepared by a film formation method. The polymer and the drug were dissolved in acetonitrile, and the organic solvent was evaporated under reduced pressure at room temperature with subsequent drying at 0.1 mbar for 1 h. Micelle formation took place upon redispersion of the resulting film with phosphate buffer (0.05 M, pH 7.4) while shaking without additional heating or sonication. Unloaded micelles and blanks were prepared according to the same procedures in the absence of Sagopilone or the polymer, respectively. The resulting dispersions were sterilized by filtration through 0.22-μm syringe filters (Millex®-GV 0.22 μm, Millipore, USA).