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Optimization of the hydrophobic domain in poly(ethylene oxide)-poly(ε-caprolactone) based nano-carriers for the solubilization and delivery of Amphotericin B

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ABSTRACT

The aim of the study was to develop a polymeric nano-carrier based on methoxy poly(ethylene oxide)-*b*-poly(ε-caprolactone) (MePEO-*b*-PCL) for the optimum solubilization and delivery of Amphotericin B (AmB). For this purpose, MePEO-*b*-PCL block co-polymers containing palmitoyl substituent on PCL (at a 100% substitution level) were synthesized through preparation of substituted monomer, that is, α-palmitoyl-ε-caprolactone, and further ring opening polymerization of this monomer by methoxy PEO (5000 g mol^{-1}) using stannous octoate as catalyst. Prepared block co-polymers were characterized for their molecular weight by ^{1}H NMR and gel permeation chromatography, and assembled to polymeric nano-carriers. The self-assembly of synthesized MePEO-*b*-PPaCL to spherical particles of nanometer size range was shown by dynamic light scattering (DLS) and transmission electron microscopy (TEM). The efficacy of nano-carriers formed from this structure (abbreviated as MePEO-*b*-PPaCL) in comparison to unmodified MePEO-*b*-PCL and those with benzyl and cholesteryl substituent on PCL (abbreviated as MePEO-*b*-PBCL and MePEO-*b*-PChCL, respectively) on the solubilization and hemolytic activity of AmB against rat red blood cells was assessed. Under identical conditions, the maximum solubilization of AmB was achieved by nano-carriers prepared from MePEO-*b*-PPaCL (436 μg/mL), followed by MePEO-*b*-PChCL (355 μg/mL), MePEO-*b*-PBCL (296 μg/mL) and MePEO-*b*-PCL (222 μg/mL). The hemolytic activity of AmB was reduced the most by its encapsulation in MePEO-*b*-PChCL nano-particles which showed only 7% hemolysis at 30 μg/mL AmB concentration. This was followed by MePEO-*b*-PCL nano-particles which illustrated 15% hemolysis, MePEO-*b*-PPaCL with 40% hemolysis and MePEO-*b*-PBCL with 60% hemolysis at 30 μg/mL AmB concentrations, respectively. In contrast Fungizone® showed 90% hemolysis at 30 μg/mL AmB concentration. Based on the improved solubility and reduced hemolytic activity, the MePEO-*b*-PChCL nano-carriers are considered as optimum structures for AmB delivery.

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1. Introduction

Amphotericin B (AmB) is the most potent, membrane active polyene macrolide antifungal agent used to treat systemic mycosis in clinical practice [1,2]. It is widely believed that AmB acts by interacting more explicitly with the ergosterol in fungal cell membrane, but it also interacts with cholesterol of the mammalian cell membrane [3,4]. Poor water solubility and severe toxicity are major drawbacks for AmB application [5,6]. Fungizone®, is the standard water soluble formulation of AmB which uses sodium deoxycholate as a solubilizating agent. Formulation and drug dependent side effects associated with the administration of Fungizone®, have restricted the clinical benefit of AmB therapy [7–9]. Alternative lipid-base AmB formulations have been developed and commercialized, that were successful in overcoming the problem of poor water solubility and dose-dependent AmB toxicity [10]. The use of these formulations is not adopted extensively in clinical practice, however, perhaps because of variability in their pharmacokinetics, infusion related side effects at elevated AmB doses, and high cost [11–14].

The objective of this study is to develop a polymer based nano-carrier that can overcome the limitations of current AmB formulations and enhance the therapeutic benefit of this potent antifungal agent in clinic [8,15–20]. Success in the development of a better formulation for AmB will be defined by the ability of the drug carrier in the solubilization of AmB and controlling its release leading to a reduction in AmB interaction and toxicity towards mammalian cells. The level of AmB solubilization and release both,

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will depend on the strength of interactions between drug and polymeric carrier

In previous studies, MePEO-*b*-poly(L-amino acid) micelles with stearyl modified core structure were tried for AmB delivery. Substitution of stearyl groups in the core of MePEO-*b*-poly(L-amino acid) micelles has shown to increase the solubility of AmB in its micellar carrier and reduce the hemolytic activity of encapsulated AmB [21,22]. AmB solubilization by original MePEO-*b*-poly(ε-caprolactone) (MePEO-*b*-PCL) nano-carriers, appears to be higher than what has been reported with MePEO-*b*-poly(L-amino acid) micelles (without stearyl modification) [23]. Besides, the MePEO-*b*-PCL nano-carriers are thermodynamically more stable than MePEO-*b*-poly(L-amino acid) micelles as reflected by a lower CMC. Therefore, MePEO-*b*-PCL nano-carriers with the right substituent on the PCL that can interact with AmB efficiently without jeopardizing micellar stability could provide an optimum carrier for AmB solubilization and delivery.

To find the right structure of the substituent, we first modified the PCL structure of MePEO-*b*-PCL nano-carriers through substitution of pendent free carboxyl groups on α Carbone of caprolactone monomers at 100% substitution level. Since AmB contains many hydroxyls in its structure (Fig. 1), it can potentially interact with COOH groups in a polymer through hydrogen bounding. Nano-carriers formed from this structure have increased the solubilization of AmB even further to what has been achieved with MePEO-*b*-PCL. However, the introduction of free carboxyl in the core structure had a negative impact on the thermodynamic stability of nano-carriers and hemolytic activity of encapsulated AmB. When 40% of carboxyl groups in the core were esterified with stearic acid, AmB solubility was still higher than what achieved with original MePEO-*b*-PCL micelles. The hemolytic activity of encapsulated AmB; however, was higher than what observed with MePEO-*b*-PCL formulation and lower than that for micelles containing 100% free carboxyls in the core [24]. The results of that study pointed to the potential of PCL modified MePEO-*b*-PCL nano-carriers in achieving an optimum formulation for AmB, but showed the necessity for further efforts in finding the right substituent on PCL.

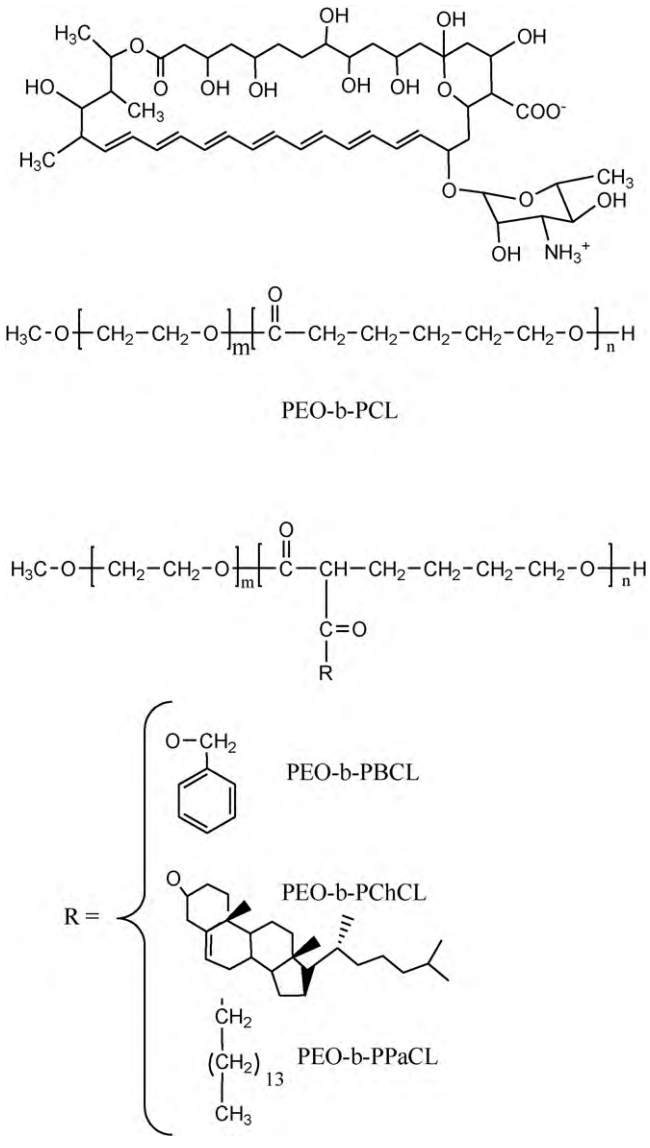

Fig. 1. Chemical structure of Amphotericin B and MePEO-*b*-poly(esters) polymers used in this study.

In this context, a new member of MePEO-*b*-PCL co-polymers family with palmitoyl substitutes at 100% substitution level on the PCL block was synthesized, and the nano-carriers formed from this structure were compared to original MePEO-*b*-PCL, those with benzyl or cholesteryl groups on the core structure (at 100% substitution level), i.e., MePEO-*block*-poly(α-benzyl-ε-caprolactone) (MePEO-*b*-PBCL) and MePEO-*block*-poly(α-cholesteryl carboxylate-ε-caprolactone) (MePEO-*b*-PChCL), respectively (Fig. 1) for the solubilization and delivery of AmB. In the biological system, AmB is carried by lipoproteins which are the carriers of cholesterol and fatty acid esters as well [25,26]. Moreover, previous research has shown the interaction of AmB with cholesterol in MePEO–DSPE/cholesterol [1] micellar structures and with acyl chains in micelles composed of MePEO-*b*-poly(amino acid)s modified with fatty acid esters in the core [27]. Owing to favorable interactions between AmB and cholesteryl or fatty acid ester, MePEO-*b*-PCL nano-carriers with these substituent structures in the core, were expected to provide better solubilization and reduced delivery of AmB to mammalian cell membrane leading to attenuated hemolytic activity when compared to original MePEO-*b*-PCL nano-carriers. The validity of this hypothesis was evaluated here.

2. Materials and methods

2.1. Materials

Amphotericin B (AmB), MePEO (average molecular weight of 5000 g mol^{-1}), diisopropylamine (99%), sodium (in kerosin), benzophenone, butyl lithium (Bu-Li) in hexane (2.5 M solution), palladium-coated charcoal, palmitoyl chloride, cholesteryl chloroformate, triton X 100 were purchased from Sigma, St. Louis, MO. Diisopropylamine was dried over calcium hydride at room temperature and freshly distilled before use. ε-Caprolactone was purchased from Lancaster Synthesis, U.K., dried over calcium hydride for 48 h at room temperature, and freshly distilled before polymerization. Tetrahydrofuran (THF) was refluxed over sodium and benzophenone for several hours and distilled immediately before use. Stannous octoate was purchased from MP Biomedicals Inc., Germany, and used without further purification. Fluorescent probe 1,3-(1,1′-dipyrenyl)propane was purchased from Molecular Probes, U.S.A. 1,2-Distearyl-sn-glycero-3-phosphocholine was from Sygnena Inc. (Cambridge, MA, USA). Phosphotungestic acid solution 10% (v/v) was purchased from Sigma–Aldrich (Oakville, ON, Canada). All other chemicals were reagent grade and were used as received.

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