Saudi Pharmaceutical Journal (2017) **25**, 258–265

King Saud University

Saudi Pharmaceutical Journal

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Development and characterization of methoxy poly (ethylene oxide)-*block*-poly(ε-caprolactone) (PEO-*b*-PCL) micelles as vehicles for the solubilization and delivery of tacrolimus

Raisuddin Ali, Abubakar Farah, Ziyad Binkhathlan *

Department of Pharmaceutics, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia

Received 12 January 2016; accepted 26 June 2016
Available online 1 July 2016

KEYWORDS

Block copolymer;
PEO-*b*-PCL;
Polymeric micelles;
Tacrolimus;
Drug delivery

Abstract Tacrolimus is a potent immunosuppressant; however, it suffers from several problems such as poor water solubility (4–12 μg/mL), low and variable oral bioavailability in patients, and narrow therapeutic window that could not be solved by the currently available *i.v.* formulation (Prograf®). Moreover, Prograf® contains HCO-60 (PEGylated castor oil) as a surfactant, which is reported to cause several side effects including hypersensitivity reactions. Therefore, the aim of the present study was to investigate the potential of PEO-*b*-PCL polymeric micelles as alternative vehicles for the solubilization and delivery of tacrolimus. Four PEO-*b*-PCL block copolymers, with different molecular weights of PCL, were synthesized by ring opening polymerization of ε-caprolactone using methoxy polyethylene oxide (5,000 g mol^{-1}) as initiator and stannous octoate as catalyst. Synthesized copolymers were characterized for their average molecular weights and polydispersity index by ^{1}H NMR and gel permeation chromatography (GPC), respectively. Drug-free micelles of PEO-*b*-PCL were prepared through a co-solvent evaporation method using acetone as the organic co-solvent. Tacrolimus-loaded micelles were prepared using the same method with different initial amounts of drug. Prepared micelles were characterized for their mean diameter size and polydispersity of the micellar population by dynamic light scattering, and an HPLC assay was used to determine the encapsulation efficiency of tacrolimus. The average molecular weights of the synthesized copolymers were in the range of 8,400–28,000 with narrow distributions (PDI = 1.06–1.11). The copolymers were designated according to the degree of polymerization of ε-caprolactone, namely PEO_{114}-*b*-PCL_{30}, PEO_{114}-*b*-PCL_{60}, PEO_{114}-*b*-PCL_{120}, and PEO_{114}-*b*-PCL_{200}. All the prepared micelles were having diameters sizes less than 100 nm with narrow distributions. The highest drug solubilization was achieved with PEO_{114}-*b*-PCL_{120}, where the

* Corresponding author.
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jsps.2016.06.009

aqueous solubility of tacrolimus exceeded 300 μg/mL. Our results show a potential for PEO-*b*-PCL micelles as solubilizing vehicles for the delivery of tacrolimus.

1. Introduction

Tacrolimus is a 23-membered macrolide isolated from *Streptomyces tsukubaensis*. It is a potent immunosuppressive agent used clinically to reduce the risk of organ rejection in postoperative transplant patients (Rath, 2013). It binds to an immunophilin, FK506 binding protein 12 and creates a new complex (Halloran, 1996). This new complex inhibits the calcineurin phosphatase which prevents the translocation of activated T-cell transcription factor and promotes proliferation of helper T-cells which results in suppression of immune response associated with tissue or organ transplant (Thomson et al., 1995).

Tacrolimus is a BCS class II with low solubility and high permeability drug. Although it is a potent immunosuppressant, however, it suffers from several problems such as poor water solubility (4–12 μg/mL), low oral bioavailability, narrow therapeutic window, and high intra- and inter-subject variability (Venkataramanan et al., 1995). Low oral bioavailability of tacrolimus is due to various factors such as low solubility, extensive first pass metabolism in liver and gut, P-glycoprotein mediated drug efflux, influence of food intake and concomitant medication (Venkataramanan et al., 1995; Tamura et al., 2002). Moreover, the currently available *i.v.* formulation of tacrolimus (Prograf®) contains HCO-60 (PEGylated castor oil) as a surfactant, which is reported to cause several side effects including hypersensitivity reactions (Nicolai and Bunyavanich, 2012; Jang et al., 2015; Hisatomi et al., 1993).

Different pharmaceutical approaches such as prodrugs, cyclodextrin intrusion complexes, liposomes, nano lipid particles, pH sensitive microspheres, and self microemulsifying drug delivery system were applied to overcome the problems associated with tacrolimus delivery (Patel et al., 2012). All of these approaches were aimed to increase the water solubility of tacrolimus in order to achieve proper absorption from the gastrointestinal tract or to minimize the pre-systemic metabolism by cytochrome P450 and inhibition of P-glycoprotein mediated efflux.

In the present study, PEO-*b*-PCL polymeric micelles were used as vehicles to overcome the solubility problem of tacrolimus, and to serve as safer alternative to the HCO-60-based formulation. Polymeric micelles are self-assemblies of synthetic amphiphilic molecules in which hydrophobic ends make the core of micelles and hydrophilic ends make the shell. Polymeric micelles are generally made up of diblock or multiblock copolymers where individual block polymers/unimers are attached to each other with non-covalent interaction. The core of a micelle is hydrophobic and it helps in solubilization of hydrophobic drugs, while the shell is hydrophilic and helps in suspending the micelles in aqueous media. The advantages of PEO-*b*-poly(ester) micelles are biocompatibility, enhanced drug solubility, controlled release, and increased blood circulation time (Aliabadi and Lavasanifar, 2006).

Methoxy poly(ethylene oxide) (PEO) and poly(ε-caprolactone) (PCL) were selected to synthesize diblock copolymer for micelle preparation. PCL is a synthetic, lipophilic, semi-crystalline, and biodegradable polymer (Dash and Konkimalla, 2012). It forms the core of micelles and helps in solubilization of lipophilic drugs. PEO is a hydrophilic polymer that makes up the shell of micelles. It also adds the stealth property to the micelles, which helps in evading the reticulo-endothelial system (RES) and improving the blood compatibility of micelles (Lin et al., 2005; Otsuka et al., 2003). PEO-*b*-PCL diblock copolymers with different molecular weights of the core-forming block were synthesized by controlled ring opening polymerization of ε-caprolactone. A series of four polymers were synthesized and characterized by nuclear magnetic resonance (NMR) spectroscopy and gel permeation chromatography (GPC). Micelles were prepared using these four diblock copolymers with different drug to polymer ratios. The prepared micelles were characterized in terms of drug loading, encapsulation efficiency, diameter size, and polydispersity index.

2. Materials and methods

2.1. Materials

Tacrolimus was extracted from expired Prograf® 5 mg capsules (Batch # 7241), as previously described (Binkhathlan et al., 2015). Methoxy PEO (M_n 5,000), stannous octoate (~95%), ε-caprolactone (97%), and THF (HPLC grade) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Acetonitrile (HPLC grade) was supplied by Fisher Scientific Co. (Leicestershire LE/15 RG, UK). Deionized water was prepared in-house using Millipore system.

2.2. Methods

2.2.1. Synthesis of PEO-b-PCL block copolymers

PEO-*b*-PCL block copolymers were synthesized by ring opening polymerization of ε-caprolactone using methoxy PEO (M_n 5,000) as an initiator and stannous octoate as a catalyst, as previously reported (Aliabadi et al., 2005). Briefly, methoxy PEO, ε-caprolactone and stannous octoate were added to a previously flamed ampoule, nitrogen purged, and then sealed under vacuum. The reaction proceeded at 140 °C for 4 h. Different ε-caprolactone to methoxy PEO feed ratios were used to synthesize PEO-*b*-PCL block copolymers with varying degrees of ε-caprolactone polymerization.

2.2.2. Characterization of the synthesized copolymers

^{1}H NMR spectrum of PEO-*b*-PCL in $CDCl_3$ at 500 MHz (Bruker Ultra shield 500.133 MHz spectrometer) was used to determine the number averaged molecular weight of the block copolymers. The degree of polymerization of ε-caprolactone was estimated by comparing the peak intensity of PEO