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Long-lasting anti-platelet activity of cilostazol from poly(ε-caprolactone)-poly(ethylene glycol) blend nanocapsules

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ABSTRACT

Cilostazol (CLZ) acts as a vasodilator and antiplatelet agent and is the main drug for the treatment of intermittent claudication (IC) related to peripheral arterial disease (PAD). The usual oral dose is 100 mg twice a day, which represents a disadvantage in treatment compliance. CLZ presents several side effects, such as headache, runny nose, and dizziness. This paper aimed to obtain novel polymeric nanocapsules prepared from poly(ε-caprolactone)-poly(ethylene glycol) (PCL-PEG) blend containing CLZ. Nanocapsules showed pH values between 6.1 and 6.3, average size lower than 137 nm, low polydispersity index (< 0.22) and negative zeta potential. These nanoformulations demonstrated spherical shape with smooth surface. Results achieved by X-ray diffraction (XRD) and differential scanning calorimetry (DSC) indicated drug amorphization compared to pure CLZ. Fourier-transformed infrared spectroscopy (FTIR) showed no chemical bonds between drug and polymers. Formulations presented suitable stability for physical parameters. The *in vitro* drug release demonstrated prolonged release with no burst effect. Drug release was controlled by both mechanisms of polymer relaxation/degradation and Fickian diffusion. Moreover, chosen CLZ-loaded nanocapsules provided an *in vivo* prolonged antiplatelet effect for CLZ statistically similar to aspirin. These formulations can be further used as a feasible oral drug delivery carrier for controlled release of CLZ in order to treat PAD and IC events.

1. Introduction

Peripheral arterial disease (PAD) is due to flow-limiting atherosclerotic plaques in lower limbs which led to intermittent claudication (IC). IC is a very painful condition usually related to symptomatic PAD that involves several symptoms as discomfort, numbness, paresthesia, cramp and fatigue of leg muscles during walking which is relieved after a few minutes of rest [1]. In addition, depressive symptoms and social isolation are also associated to IC due to restricted mobility [2]. IC is a relatively common condition; its frequency increases dramatically with age and significantly higher rates of occurrence are seen in older adults. The prevalence of IC among people aged 45–54 years is 0.6% and 55–74 years is 4.6%. Eighteen percent population over 70 years of age have IC, with smokers and diabetics at higher rate of 50–75%, and the amputation risk is approximately 1% a year. IC is more common among men than women and among individuals with other manifestations of atherosclerosis [3]. Drug therapy for patients with IC have two separate but complementary objectives: (1) symptom relief, *i.e.* improved walking distance and quality of life, and (2) prevention of secondary vascular complications, mainly plaque rupture leading to acute thrombotic events that may be limb-threatening and/or life-threatening [4].

Cilostazol (CLZ), 6-[4-(1-cyclohexyl-1*H*-tetrazol-5-yl) butoxy]-3,4-dihydro-2(1*H*)-quinolinone, is highly recommended in the symptomatic treatment of IC in the absence of heart failure [5]. CLZ is an inhibitor of phosphodiesterase III, whose action causes an increase in intracellular cyclic adenosine monophosphate (cAMP), leading to arterial smooth muscle dilatation, increased nitric oxide signaling, and, to a lesser extent, decreased platelet aggregation [6]. Additional pleiotropic effects include increased HDL levels and lower triglyceride levels [7]. In a pooled analysis of 9 randomized clinical trials, cilostazol (100 mg taken twice daily) was associated with an improvement in maximal walking distance from baseline at 20 weeks compared with placebo and an absolute improvement of 42 m [8]. Notably, these effects were found to be

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independent of age, gender, smoking status, diabetes, PAD duration, prior myocardial infarction, and beta-blocker use. However, treatment discontinuation is unfortunately quite common; it has been estimated that up to 60% of patients stop taking cilostazol by 36 months because of its side effects [9]. Side effects as headache, diarrhea, dizziness, palpitations, and tachycardia are remarkable usual and they are possibly related to the increased intracellular level of cAMP [10]. Because of a similar mechanism of action to the inotropic agents amrinone and milrinone, which demonstrated an adverse effect on mortality in advanced heart failure, the US Food and Drug Administration has established a boxed warning for cilostazol, prohibiting its use in patients with congestive heart failure [11].

Based on its poor solubility and high permeability in gastrointestinal (GI) tract, CLZ is classified as a class II drug by the Biopharmaceutics Classification System (BCS), which indicates that its absorption is dependent on dissolution rate [12,13]. For these drugs, the low drug solubility can affect the rate and extent of absorption which can compromise bioavailability [14]. In this context, CLZ absorption in the gastrointestinal tract is usually slow, variable, and incomplete [15]. Therefore, various formulation strategies have been applied to overcome this problem focused on providing immediate or controlled release pattern. Inclusion complex between

β-cyclodextrin and CLZ was prepared by lyophilization and showed increased apparent solubility and improved dissolution rate compared to pure drug [16]. In a similar study, inclusion complex between dimethyl-β-cyclodextrin and CLZ was obtained by co-precipitation and significantly increased dissolution and absorption rate compared to Pletoz® tablets (cilostazol 50 mg) and pure CLZ [17]. A CLZ nanosuspension was prepared by the anti-solvent and high-pressure homogenization method and then sprayed to dry powders. The bioavailability of CLZ tablets prepared using spray-dried nanosized crystalline powder after oral administration to beagle dogs was markedly increased compared to that produced by nanosized tablets and commercial tablets [18]. A lipid based self-nanoemulsifying formulation of CLZ was developed by a 2^3 full factorial design and showed remarkable increase in equilibrium solubility and dissolution efficiency when compared to pure CLZ [19]. Solid dispersions containing CLZ were developed by hot-melt and thermal adhesion granulation. The relative solubility was almost three-fold higher than that of Pletaal® tablets (cilostazol 50 mg) in compositions containing D-α-tocopheryl polyethylene glycol succinate and vitamin E as excipients, in which a controlled drug release pattern was demonstrated [12]. Self-nanoemulsifying drug delivery systems containing CLZ were prepared by sonication and demonstrated improved *in vitro* drug dissolution and *in vivo* oral and parenteral bioavailability [15]. An amorphous solid dispersion containing CLZ, povidone, hydrogenated vegetable oil, and sodium carboxymethyl cellulose was prepared by spray-drying and demonstrated a controlled release and absence of drug recrystallization [20]. Spray-dried solid dispersion containing Eudragit® L100 and Eudragit® S100 also provided a controlled release of CLZ [21].

To the best of our knowledge, there is no previous report in literature concerning the nanoencapsulation of CLZ into poly(ε-caprolactone)-poly(ethylene glycol) (PCL-PEG) blend nanocapsules. Nanocapsules are defined as nanovesicular systems characterized by a polymer shell surrounding a lipophilic or hydrophilic liquid core [22]. The central cavity is usually composed of oil [23]. Preferably, the drug is entrapped or dissolved in the liquid core. However, adsorption on the particle surface can also occur [24]. Compared to nanospheres, which is arranged as a polymeric matrix, nanocapsules have been proven to be more advantageous due to their higher drug loading capacity, better protection of drugs from degradation, and reduced burst release [24–26]. In addition, as briefly described, previous works were devoted to discuss formulation parameters, drug solubility, dissolutions behavior, and bioavailability of CLZ from those systems. No other paper was focused on using the novel controlled delivery strategy in order to investigate *in vivo* pharmacological effect of CLZ.

Taking into account all these data, the hypothesis of this paper was that nanoencapsulation of CLZ in PCL-PEG blend nanocapsules can both decrease oral drug dosage and prolong *in vivo* anti-platelet effect in order to minimize its drawbacks in pharmacotherapy. A PCL-PEG blend was chosen for obtaining nanocapsules due three advantages: (1) lower cost than other polyesters as poly(lactic acid) (PLA) and poly(lactic-*co*-glycolic acid) PLGA; (2) PEGylated systems often reduce immunogenicity and antigenicity and provide a prolonged blood effect; (3) computational simulation successfully predicted suitable experimental drug affinity and drug loading for polyester-PEG systems containing CLZ [27]. At first, CLZ-loaded nanocapsules were prepared by interfacial deposition of preformed polymer method and entirely characterized by physicochemical, morphological, spectroscopic and thermal methods. Then, encapsulation efficiency and *in vitro* release profiles were determined by a previously validated reversed-phase high-performance liquid chromatography coupled to ultraviolet (RP-HPLC/UV) method. Moreover, the chosen formulation was evaluated for *in vivo* anti-platelet activity in rats.

2. Material and methods

2.1. Materials

Cilostazol 99.91% purity (CLZ, IPCA Laboratories Limited, Mumbai, India), poly(ε-caprolactone) (PCL, $\overline{Mw}$= 10,000–14,000 g mol^{-1}, Sigma-Aldrich, St. Louis, MO, USA), and poly(ethylene glycol) (PEG, $\overline{Mw}$= 5400–6600 g mol^{-1}, Cromato Produtos Químicos, Diadema, Brazil) were used as received. Span 80® (sorbitan monooleate) was purchased from Sigma Aldrich (St. Louis, MO, USA) and Tween 80® (polysorbate 80) was supplied by Deleware (Porto Alegre, Brazil). Capric/caprylic acid triglycerides (MCT) were obtained from Focus Química (São Paulo, Brazil) and acetone was acquired from Vetec (Rio de Janeiro, Brazil). HPLC-grade acetonitrile and HPLC-grade methanol were provided by Sigma-Aldrich (St. Louis, MO, USA). Water was purified in a Milli-Q Plus water purification system (Millipore, Bedford, MA, USA). All others reagents and solvents were of analytical grade.

2.2. Methods

2.2.1. Preparation of polymeric nanocapsules

CLZ-loaded nanocapsules were obtained using a blend of PCL-PEG. These formulations were prepared by interfacial deposition of preformed polymer method as classically described [28]. A colloidal solution of PCL-PEG (100 mg, 3:1 ratio), CLZ (30; 60 and 120 mg), MCT (300 mg) and Span 80® (77 mg) in acetone (27 mL) was added into an aqueous solution (53 mL) of Tween 80® (77 mg) under magnetic stirring at 40 °C. After 60 min, the acetone was removed and the aqueous phase was concentrated under reduced pressure. The final volume was adjusted to 10 mL and CLZ concentration of 3.0 mg mL^{-1} (NC3), 6.0 mg mL^{-1} (NC6) and 12.0 mg mL^{-1} (NC12). A formulation containing no drug (NC0) was prepared as negative control. All polymeric nanocapsule suspensions were obtained in triplicate from three different batches.

2.2.2. Physicochemical characterization

The pH values of nanosuspensions were determined by immersion of the electrode directly in the formulation using a previously calibrated potentiometer (pH meter model HI 221, Hanna Instruments, São Paulo, Brazil). Measures were performed at room temperature (25 ± 2 °C) in triplicate.

The average particle size and polydispersity index (n = 3) were measured by photon correlation spectroscopy (Zetasizer Nanoseries, Malvern Instruments, Malvern, UK) after diluting each sample in ultrapure water (1:500, v/v) with no previous filtration. At the same equipment and using the same sample preparation, zeta potential (n = 3) was determined by electrophoretic mobility technique [29].