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Poly(ε-caprolactone), Eudragit® RS 100 and poly(ε-caprolactone)/Eudragit® RS 100 blend submicron particles for the sustained release of the antiretroviral efavirenz

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ABSTRACT

The design of simple and scalable drug delivery systems to target the central nervous system (CNS) could represent a breakthrough in the addressment of the HIV-associated neuropathogenesis. The intranasal (i.n.) route represents a minimally invasive strategy to surpass the blood–brain barrier, though it demands the use of appropriate nanocarriers bearing high drug payloads and displaying sufficiently long residence time. The present work explored the development of submicron particles made of poly(ε-caprolactone) (PCL), Eudragit® RS 100 (RS a copolymer of ethylacrylate, methylmethacrylate and methacrylic acid esterified with quaternary ammonium groups) and their blends, loaded with the first-choice antiretroviral efavirenz (EFV) as an approach to fine tune the particle size and the release kinetics. Particles displaying hydrodynamic diameters between 90 and 530 nm were obtained by two methods: nanoprecipitation and emulsion/solvent diffusion/evaporation. In general, the former resulted in smaller particles and narrower size distributions. The encapsulation efficiency was greater than 94%, the drug weight content approximately 10% and the yield in the 72.5–90.0% range. The highly positive surface (>+30 mV) rendered the suspensions physically stable for more than one month. *In vitro* release assays indicated that the incorporation of the poly(methacrylate) into the composition reduced the burst effect and slowed the release rate down with respect to pure poly(ε-caprolactone) particles. The analysis of the release profile indicated that, in all cases, the kinetics adjusted well to the Higuchi model with R^2_{adj} values >0.9779. These findings suggested that the release was mainly controlled by diffusion. In addition, when data were analyzed by the Korsmeyer–Peppas model, *n* values were in the 0.520–0.587 range, indicating that the drug release was accomplished by the combination of two phenomena: diffusion and polymer chain relaxation. Based on ATR/FT-IR analysis that investigated drug/polymer matrix interactions, the potential role of the hydrophobic interactions of C—F groups of EFV with carbonyl groups in the backbone of PCL and poly(methacrylate) could be ruled out. The developed EFV-loaded particles appear as a useful platform to investigate the intranasal administration to increase the bioavailability in the CNS.

1. Introduction

The Human Immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS) is the most deadly infectious disease of our times with approximately 35 million infected people worldwide [1]. The High Activity Antiretroviral Therapy (HAART) has improved the therapeutic outcomes [2,3] and the disease has become chronic in most of the developed countries [4]. To ensure therapeutic success, patients need to adhere strictly to the administration schedule [5]. On the other hand, HAART does not eradicate the virus from the host due to the generation of intracellular and anatomical reservoirs, where the virus remains in latency and less accessible to antiretrovirals (ARVs) [6–8].

Blood–tissue barriers protect specific body compartments by constraining the passage of drugs owing to the activity of a variety of efflux pumps belonging to the ATP-binding cassette superfamily (ABC) [9,10], this phenomenon often resulting in reduced bioavailability [11]. ARVs are substrates of, at least, one pump [12–14]. ABCs are profusely distributed in the blood–brain barrier (BBB) [15] and they play a key role in the generation of the HIV reservoir in the central nervous system (CNS) [16,17]. In CNS, the virus leads to a gradual deterioration of the cognitive functions, a disease known as

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HIV-associated neurocognitive disorder (HAND) [18–20] that hits especially younger patients [21]. The design of simple and effective drug delivery systems that effectively target the CNS could represent a breakthrough to tackle the HIV neuropathogenesis [18,22].

Different nanocarriers are being explored to passively or actively target ARVs to the CNS [23,24]. Some works employed nanoparticles surface-decorated with ligands that are recognizable by specific receptors in the apical surface of the BBB [25]. Other research groups co-administered ARVs with different poly(ethylene oxide)–poly(propylene oxide) (PEO–PPO) amphiphiles [26] that inhibit the functional activity of ABCs and improve the bioavailability of the drug in the CNS [27,28]. Gendelman and coworkers designed an interesting cell delivery platform employing drug-loaded "macrophage ghosts" [29]. These approaches usually comprised the intravenous (i.v.) administration of the drug-loaded system.

The intranasal (i.n.) route capitalizes on the direct nose-to-brain transport that would involve the terminals of olfactory neurons present in the nasal mucosa [29–31]. The most appealing features of this administration route in HIV would be (i) minimal invasiveness, (ii) painlessness and (iii) possible self-administration [32]. Interestingly, the transport has been shown to be more effective for drugs encapsulated within submicron carriers than for drugs in solution, suggesting the involvement of active cell uptake pathways. A main limitation that precluded the translation of the i.n. route into clinics is the small volume that can be instilled in the nostril [33]. In this context, only systems containing great drug payloads would enable the attainment of sufficiently high doses.

Only a few works assessed the i.n. administration of ARV-loaded particles to target the CNS [34]. Recently, we compared the pharmacokinetics in plasma and CNS of efavirenz (EFV) encapsulated within single and mixed poloxamer and poloxamine polymeric micelles after i.n. and i.v. administration [35]. The bioavailability in the brain and the relative exposure index were increased four and five times, respectively, with respect to the systems administered i.v.; the relative index was calculated by taking the ratio between the area-under-the-curve in CNS and plasma. However, polymeric micelles could not sustain the release in the long-term range [35]. To achieve more prolonged release profiles that would ensure constant drug concentrations, a different nanocarrier has to be engineered.

Poly(ε-caprolactone) (PCL) is a highly hydrophobic and semicrystalline polyester that owing to its proven biocompatibility, biodegradability and permeability has found broad application in the development of drug delivery systems [36–39]. PCL has already obtained approval by the USA-Food and Drug Administration and the European Medicines Agency [40]. PCL undergoes hydrolysis and subsequent conversion into 6-hydroxylcaproic acid and acetyl-CoA *in vivo*, finally entering the citric acid pathway. *In vitro* assays in water showed that PCL degrades very slowly, though the degradation kinetics depends on its molecular weight and the size and the surface area of the implants [41,42]. Moreover, specific enzymes such as lipase catalyzed the *in vitro* degradation in approximately 1000-times [43]. The *in vivo* degradation was even faster [44].

Eudragit® comprises a series of biocompatible copolymers often used for film coating of solid formulations and the different derivatives have been accepted the regulatory agencies of USA, Europe and Japan for oral and topical administration [45]. Even though these copolymers are not biodegradable, several research groups employed Eudragit®-made nanoparticles for the parenteral administration of drugs and they reported on the good biocompatibility of this biomaterial also by these routes [46–48], owing to the rapid clearance from the systemic circulation by the mononuclear phagocytic system and their deposition in the liver [49]. Moreover, they have been used to develop tissue engineering scaffolds [50].

As a preamble to a comprehensive study of the key parameters that govern the absorption process from the olfactory mucosa (*e.g.*, particle size and composition), in the present work, we developed submicron particles made of PCL of two different molecular weights, a water-insoluble/water-permeable poly(methacrylate) (Eudragit® RS 100) and their blends loaded with the first-line ARV efavirenz (EFV). This polymer composition enabled the fine tuning of the particle size and the release profile (especially the burst effect) with respect to a control of pure PCL.

2. Experimental

2.1. Materials

Poly(ε-caprolactone) of molecular weight 14,000 g/mol (PCL_L) was purchased from Sigma–Aldrich (USA). A highly hydrophobic PCL diol (PCL_H, M_{nGPC} = 40,400 g/mol; M_{wGPC} = 64,200 g/mol) was synthesized by the microwave-assisted ring-opening polymerization of ε-CL (CL, Sigma–Aldrich) initiated by poly(ethylene glycol) (PEG, molecular weight 400 g/mol, Sigma–Aldrich) and catalyzed by tin(II) 2-ethylhexanoate (SnOct, Sigma–Aldrich) [51]. The PEG content in this copolymer was below 1% in weight, thus resulting in a copolymer with the intrinsic properties of pure PCL [51]. Eudragit® RS 100 (RS, powder, a copolymer of ethylmethacrylate, methylmethacrylate and methacrylic acid esterified with quaternary ammonium groups) and Pluronic F68 were kind gifts of Evonik (Argentina) and BASF (USA), respectively. EFV was a donation of LKM Laboratories (Argentina). KH_2PO_4, NaOH, Tween® 80 and solvents were of analytical grade and used as received. Acetonitrile (ACN, HPLC grade, Sintorgan, Argentina) was used as mobile phase in liquid chromatography analyses (see below).

2.2. Preparation of EFV-loaded submicron particles

EFV-loaded submicron particles were produced by two methods: (i) nanoprecipitation and (ii) simple oil-in-water (o/w) emulsion and solvent diffusion/evaporation. Regardless of the fact that some of the systems described in the present study were submicron in size, those obtained by precipitation were usually in the 1–200 nm size range.

Nanoprecipitation. PCL and Eudragit® RS 100 (1:1 and 1:3 weight ratio, 60 mg total weight) were suspended in acetone (10 mL) and gently heated at 37 °C and stirred, until complete dissolution. Then, EFV (6 mg) was added and thoroughly mixed for 15 min. This solution was poured into a syringe (10 mL) and injected with a needle (21G1, 0.80 mm × 25 mm) on distilled water (20 mL) containing Pluronic F68 (60 mg) at a constant flow rate (20 mL/h, infusion pump PC11U, APEMA, Argentina) under moderate magnetic stirring and at room temperature. The aqueous phase played the role of antisolvent and favored the precipitation of the polymeric particles. The resulting suspension was stirred for 3 h to allow the complete evaporation of the organic solvent and filtered under vacuum through filter paper. Then, samples were frozen at −20 °C and lyophilized (Freeze Dryer Unit GAMMA A, CHRIST®, Germany) for 48 h. The EFV payload was determined by high performance liquid chromatography (HPLC) (see below). Products were stored at room temperature protected from light and moisture until use. Pure PCL and Eudragit® RS 100 particles were also produced as described above. Blank particles (without the incorporation of drug) were used as controls. The different production conditions used are summarized in Table 1.

Simple oil-in-water (o/w) emulsion and solvent diffusion/evaporation. Aiming to assess the effect of the solvent on the size and size distribution, two solvents were used. In brief, PCL:Eudragit® RS 100 blends (1:1 weight ratio, 60 mg total weight) were dissolved in dichloromethane (DCM, 5 mL) or ethyl acetate