International Journal of Pharmaceutics 506 (2016) 116–128

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Oral delivery of camptothecin using cyclodextrin/poly(anhydride) nanoparticles

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ARTICLE INFO

Article history:
Received 4 March 2016
Received in revised form 15 April 2016
Accepted 16 April 2016
Available online 19 April 2016

Chemical compounds studied in this article:
Camptothecin (PubChem CID: 24360)
Gantrez AN (PubChem CID: 62708)
2-Hydroxypropyl-β-cyclodextrin (PubChem CID: 44134771)
β-Cyclodextrin (PubChem CID: 444041)
Methyl-β-cyclodextrin (PubChem CID: 51051622)

Keywords:
Camptothecin
Nanoparticles
Cyclodextrins
Oral delivery

ABSTRACT

Camptothecin (CPT), a molecule that shows powerful anticancer activity, is still not used in clinic due to its high hydrophobicity and poor active form's stability. In order to solve these drawbacks, the combination between poly(anhydride) nanoparticles and cyclodextrins was evaluated.

CPT-loaded nanoparticles, prepared in the presence of 2-hydroxypropyl-β-cyclodextrin, (HPCD-NP) displayed a mean size close to 170 nm and a payload of 50 μg per mg (25 times higher than the one of the control nanoparticles). CPT was not released from nanoparticles under gastric conditions. However, under intestinal conditions, about 50% of the drug content was released as a burst, whereas the remained drug was released following a zero-order kinetic. Pharmacokinetic studies revealed that the CPT plasma levels, from orally administered nanoparticles, were high and sustained up to 48 h. The CPT oral bioavailability was 7-fold higher than the value obtained with the control, whereas its clearance was significantly lower than for the aqueous suspension. These observations may be directly related to a prolonged residence time of nanoparticles in close contact with the intestinal epithelium, the presence of the cyclodextrin that decreases the CPT transformation into its inactive form and the generation of an acidic microenvironment during the degradation of the poly(anhydride) that would prevent the transformation of the active lactone into the inactive carboxylate conformation.

1. Introduction

Oral chemotherapy is an attractive approach for cancer treatment because of its convenience, safety and patient acceptance, especially in chronic regimens (Borner et al., 2001; O'Neill and Twelves, 2002). In recent studies, patients valued positively and expressed their preference for the oral route of administration since it interferes less with their daily life, giving them a feeling of freedom and a better quality of life (Halfdanarson and Jatoi, 2010). Furthermore, oral administration avoids the discomfort of injection and can be eventually conducted at home. Unfortunately, for a number of anticancer drugs, their oral administration remains a challenge. This fact would be directly related to inadequate physico-chemical and stability properties as well as to the physiological barriers, which dramatically hamper the adequate absorption of such drugs.

20(*S*)-camptothecin (CPT) is a cytotoxic quinoline alkaloid which possess an affinity to the DNA enzyme topoisomerase I. The activity of CPT would be mediated by its binding to this enzyme during S-phase, leading to the accumulation of single DNA strands, and thus, to cell death (Li et al., 2006). Nevertheless its therapeutic application was (and remains to be) hindered by a very low solubility in aqueous media, high toxicity, and rapid inactivation through lactone ring hydrolysis at physiological pH conditions. In fact, CPT exists in a dynamic equilibrium between the closed-ring lactone moiety and the open-ring carboxylic acid form (Fig. 1). The first form (lactone) is responsible for the molecule's powerful anticancer activity, whereas the carboxylate form shows a reduced efficacy associated with a high toxicity (Lorence and Nessler, 2004). These two forms coexist at 50% at a pH of 6.65, being the equilibrium moved towards the lactone form at lower pH and favoring the carboxylate open ring at higher pH (Wall et al., 1966). Regrettably, the open-ring form, predominant at physiological pH shows less than 10% potency of the closed-ring form as

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http://dx.doi.org/10.1016/j.ijpharm.2016.04.045
0378-5173/

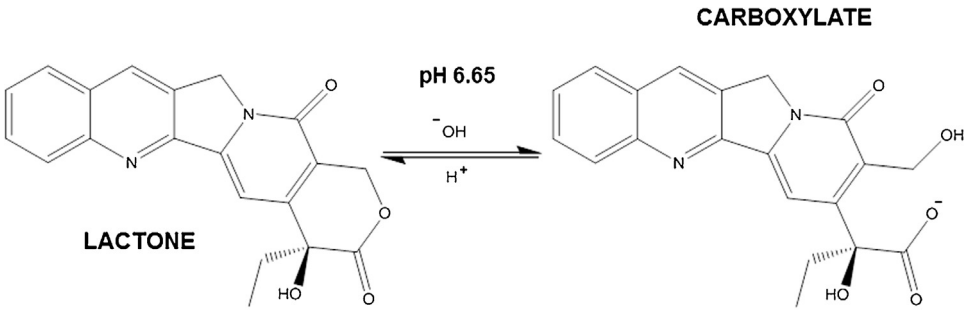

Fig. 1. Chemical structure of camptothecin in its lactone form and carboxylate conformation.

topoisomerase I inhibitor (Sriram et al., 2005). This fact would be related to the high affinity of the carboxylate form for human serum albumin, making it inaccessible for cellular uptake (Sun et al., 2012).

In order to solve these drawbacks, CPT derivatives, such as topotecan and irinotecan were synthesized. Both were approved for clinical use in Japan, Europe and USA (Oberlies and Kroll, 2004). Although they offer the advantage of their better water solubility, camptothecin still shows lower IC50 against a variety of cancer cell lines (Thomas et al., 2004). More recently, other approaches based on the use of drug delivery systems have been also proposed including nanoparticles (Min et al., 2008), liposomes (Watanabe et al., 2008), micelles (Kawano et al., 2006), polymer derivatives (Weiss et al., 2013), and solid lipid nanoparticles (Yang et al., 1999; Martins et al., 2013).

Another possible strategy would be the incorporation of CPT in poly(anhydride) nanoparticles made from the copolymer of methylvinylether and maleic anhydride. In the recent past, these nanoparticles have demonstrated an important capability to develop adhesive interactions with the gut epithelium and improve the oral bioavailability of different drugs such as fluorouridine (Arbos et al., 2004), paclitaxel (Agüeros et al., 2010; Zabaleta et al., 2012) and atovaquone (Calvo et al., 2011). In addition, these nanoparticles can be easily combined with different ligands and excipients in order to improve their mucus-permeating properties and/or to modify their distribution within the gastrointestinal tract (Arbos et al., 2002; Calleja et al., 2014). In this particular work, the combination of these poly (anhydride) nanoparticles with cyclodextrins has been studied. This combination may offer interesting advantages. First, the use of cyclodextrins facilitates the encapsulation of lipophilic drugs in polymeric nanoparticles increasing the resulting payload (Agüeros et al., 2010; Calvo et al., 2011). Second, the presence of cyclodextrins modulates and sustains the release of lipophilic compounds from polymeric nanoparticles (Agüeros et al., 2009; Penalva et al., 2015). Third, in the particular case of camptothecin, cyclodextrins may improve up to 10 times the lactone form's half-life (Kang et al., 2002). In addition, some cyclodextrins (*i.e.*, 2-hydroxypropyl-β-cyclodextrin) display an inherent ability to inhibit different enzymatic complexes and extrusion pumps localized in the intestinal epithelium (Ishikawa et al., 2005; Zhang et al., 2011).

Therefore, the aim of this work was to optimize the preparative process of camptothecin-loaded poly(anhydride) nanoparticles combined with cyclodextrins as well as to evaluate the *in vitro* properties and *in vivo* capabilities of the developed nanoparticles to promote the oral absorption and bioavailability of this drug in Wistar rats.

2. Materials and methods

2.1. Reagents

Poly(methyl vinyl ether-co-maleic anhydride) or poly(anhydride) (PMV/MA) [Gantrez® AN 119; MW 200,000] was purchased from ISP (Barcelona, Spain). Camptothecin (CPT) (99.0%) was supplied by 21CECpharm (London, UK). β-Cyclodextrin (β-CD) and 2-hydroxypropyl-β-cyclodextrin (HP-β-CD), pepsin and pancreatin were obtained from Sigma Aldrich (Germany) whereas methyl-β-cyclodextrin (M-β-CD) and sulfopropyl-β-cyclodextrin (SP-β-CD) were from Cyclolab (Hungary). Acetone, ethanol, acetonitrile and trifluoroacetic acid (TFA) were obtained from Merck (Darmstadt, Germany). Deionized reagent water was prepared by a water purification system (Wasserlab, Spain). All reagents and chemicals used were of analytical grade.

2.2. Solubility studies

CPT solubility studies were carried out according to the Higuchi and Connors method (Higuchi and Connors, 1965). An excess of CPT was added to a deionised aqueous solution in vials containing increasing amounts of the oligosaccharide (β-CD, HP-β-CD, M-β-CD or SP-β-CD). These flasks were sonicated for 5 min, sealed and shaken in a VorTemp 56™ Shaking Incubator (Labnet International Inc., USA) at 25 °C for 72 h. Then, samples were filtered (0.45 μm) and the concentration of CPT was determined by HPLC (see Section 2.4.2). The presence of trace amounts of cyclodextrins did not interfere with the assay. The assays were performed in triplicate.

The apparent stability constants (K_c) and the stoichiometry of the camptothecin-cyclodextrin complexes (CPT-CD) were estimated from the phase solubility diagrams. For A_L diagrams, the apparent stability constant (K_c) of the drug-oligosaccharide complex can be calculated as follows (Loftsson et al., 2004):

$$K_c = \frac{\text{slope}}{S_0\,(1 - \text{slope})} \quad (1)$$

where S_0 is the molar solubility of camptothecin in absence of cyclodextrins and the slope is obtained from the initial straight-line portion of the plot of camptothecin concentration against the cyclodextrin concentration.

Moreover, the complexation efficiency (CE) was calculated from the slope of the linear phase of the phase-solubility diagram (Loftsson et al., 2005):

$$CE = S_0 K_c = \frac{\text{slope}}{1 - \text{slope}} \quad (2)$$