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Nano-precipitated curcumin loaded particles: effect of carrier size and drug complexation with (2-hydroxypropyl)-β-cyclodextrin on their biological performances

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ABSTRACT

In this work, curcumin (CURC)-encapsulating nanoparticles (NPs), made up of an amphiphilic blend of poloxamers and PLGA (PPC NPs) at different polymer concentrations, were prepared by nano-precipitation. CURC was preliminarily complexed with (2-hydroxypropyl)-β-cyclodextrin (HPβCD) to improve its loading efficiency. The formation of host-guest complexes of CURC with HPβCD (CD-CURC) was confirmed by means of ^{1}HNMR studies and differential scanning calorimetry (DSC). Nano-precipitation allowed to obtain NPs with a small size (90–120 nm depending on the polymer concentration), a narrow size distribution and stable in water for 30 days at 4 °C and in RPMI-1640 cell culture medium up to 72 h at 37 °C. The *in vitro* release of CD-CURC, sustained up to 5 days, was governed mainly by a diffusive mechanism. It was also found that the produced NPs were efficiently internalized by mesothelioma cells (MSTO-211H) in the cytoplasmic space, at an extent strongly dependent on NP size and polydispesity index, therefore pointing at the importance of NP preparation method in improving their uptake.

1. Introduction

Curcumin (CURC) is a naturally occurring polyphenol found in the rhizome of *Curcuma Longa Linn*. It is considered a nutraceutical and has been used for different pharmaceutical applications, such as anti-inflammatory, antimicrobial, antioxidant and anticancer. The use of CURC is also promising due to its safety and intrinsic non-toxicity to humans, even at high doses (Lao et al., 2006; Mehanny et al., 2016). Over the last decades, the activity of CURC in cancer prevention and treatment has been widely recognized and is known to be mediated by multiple signaling pathways (Mandal et al., 2009). In addition, the specific cytotoxic effect of CURC against tumors, along with its inhibitory effects on protein kinase C and Epidermal Growth Factor Receptor tyrosine kinase (EGFR), have been demonstrated in various human cancer cell lines (Dorai et al., 2000). Furthermore, CURC has been demonstrated to induce cell cycle arrest and/or apoptosis by blocking the activity of Nuclear Factor kappa B (NFκ-B), which is involved in the proliferation of cancer cells (Kotecha et al., 2016). It should also be highlighted that a constantly increasing number of clinical trials of CURC have been

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published or are currently in progress, therefore evidencing the flourishing research interest of the scientific community on the therapeutic potential of CURC (Hatcher et al., 2008).

Unfortunately, *in vivo* bioavailability of CURC is seriously curtailed owing to its poor aqueous solubility, photodegradation, chemical instability, and rapid metabolism. Therefore, aiming to ameliorate the therapeutic efficacy and bioavailability profile of CURC, numerous attempts have been made to properly design suitable nanocarriers for CURC delivery and targeting (Ghosh and Ryan, 2014; Martin et al., 2015; Mathur and Gupta, 2010; Mohanty et al., 2010; Niskanen et al., 2016; Peng and Qian, 2014; Sun et al., 2012; Tang et al., 2010).

Actually, nanosized devices are regarded as the breakthrough frontier of materials science and are becoming increasingly important since they offer the chance to boost the biological effect of CURC by increasing its *in vivo* half-life, modifying its pharmacokinetic profile and reducing its release to non-target tissues. In particular, in a recent study, it has been shown that the IC50 of CURC-loaded polymeric micelles was as low as few μg/mL for breast carcinoma cells and their drug-resistant analogue (Zhao et al., 2012). This outcome has been attributed to the small size of the micelles (approximately 70 nm), to the increased CURC stability and solubility within the micelles, and to their higher uptake due to the presence of superficial poloxamer.

In a previous work, we have produced CURC-loaded NPs made of a polylactic-*co*-glycolic acid (PLGA)/poloxamers blend (namely PP NPs) by the double emulsion-solvent evaporation technique. The obtained NPs were found to expose superficial hydrophilic moieties, which endowed the devices with a prolonged dimensional stability both in water and in cell culture medium. Furthermore, the NPs were able to induce an enduring block in the G0/G1 phase of mesothelioma cell cycle, up to 72 h, therefore overtaking drug tolerance, which generally arises when free CURC is administered (Mayol et al., 2015). However, the effectiveness of this phenomenon can be probably enhanced when NP uptake is optimized. In particular, NP internalization faces a size threshold limitation and, for this reason, in this study we have explored the nanoprecipitation technique with the aim to obtain NPs smaller in size and narrower in size distribution, and thus more suitable for cell uptake. To this aim, we have produced PP NPs loaded with both bare CURC and with the inclusion complex formed by CURC and 2-hydroxypropyl-β-cyclodextrin. Cyclodextrins are cyclic oligosaccharides, possessing a central hydrophobic cavity and a hydrophilic outer surface, which are widely used as solubility enhancers of sparingly soluble molecules (Brewster and Loftsson, 2007; Loftsson and Duchene, 2007; Miro et al., 2013; Moyano-Mendez et al., 2014). Taking advantage of their frusto-conical architecture, cyclodextrins are able to form inclusion complexes with sparingly soluble CURC, consequently improving its solubility in water and stability (Yadav et al., 2009; Yallapu et al., 2010). Thereafter, the obtained NPs have been characterized for their technological features, stability in water and in cell culture medium, cell cytotoxicity and uptake. In addition, the formation of the host-guest complex between CURC and 2-hydroxypropyl-β-cyclodextrin (HPβCD; CD in the following) has been studied in detail by means of ^{1}HNMR and differential scanning calorimetry (DSC).

2. Materials and methods

2.1. Materials

Poloxamer F127 (a = 100 and b = 65) and F68 (a = 76 and b = 29) were obtained from Lutrol (Basf, Germany) and equimolar uncapped poly(D,L-lactic-*co*-glycolic acid) (PLGA) (Resomer RG504H, Mw 40 kDa, inherent viscosity: 0.16–0.24 dL g^{-1} in acetone at 25 °C) was purchased from Evonik (Germany). CURC ((*E*,*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, purity >90%) from Cayman Chemical Company (USA), ascorbic and citric acid from J-Baker (USA) and potassium chloride (KCl) from Carlo Erba (Italy) were used. Ethanol (EtOH), acetone, dimethylsulfoxide (DMSO), 2-hydroxypropyl-β-cyclodextrin (HPβCD; CD in the text), Tween-80, dibasic sodium phosphate (Na_2HPO_4), sodium chloride (NaCl), 1,6-diphenyl-1,3,5-hexatriene (DPH), Trypan blue, propidium iodide (PI) and RNase were obtained from Sigma-Aldrich (USA). Human mesothelioma cell line MSTO-211H was obtained from the American Type Culture Collection (Rockville, MD, USA); RPMI-1640 (Roswell Park Memorial Institute) medium, supplemented with 10% Fetal Bovine Serum (FBS), penicillin/streptomycin 10 UI/mL, trypsin-ethylenediamine tetra-acetic acid (Trypsin–EDTA), 1 mM sodium pyruvate and 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) from Euroclone (Milan, Italy) were employed in a humidified incubator at 37 °C and 5% CO_2. All chemicals and media were used as received without further purification.

2.2. Preparation of curcumin-HPβCD inclusion complex

The CD/CURC inclusion complex was prepared by dissolving 1 mg of CURC and 8 mg of CD in 1 mL of EtOH. The resulting solution was stirred for 1 h at 70 °C for EtOH evaporation and, afterwards, 1 mL of double-distilled water (DDW) was added and evaporated under stirring. Finally, 2 mL of DDW were added and the obtained solution was centrifuged (10,000 rpm, 15 min). The supernatant was freeze-dried at −60 °C (24 h, 0.01 atm; Modulyo, Edwards, UK) and the obtained dried mass stored at 4 °C.

2.3. Preparation of nanoparticles

Different NP formulations were prepared using nanoprecipitation technique (Menon et al., 2012; Zeng et al., 2013). As a first step, the NPs were loaded with free CURC (*i.e.*, not complexed with CD). To prepare NPs, 5 mL of an organic phase (O) composed of PLGA/F68/F127 (1:0.5:0.5 mass ratio) solution in acetone and containing solubilised CURC (0.2% w/v) were forced through the needle of a syringe (inner diameter: 11.99 mm) at 333.3 μL/min flow rate, and poured into 40 mL of an aqueous phase (W1) containing F127 and F68 as surface active agents (1:1 w/w ratio; 0.375 mg/mL overall concentration). Three formulations of CURC-loaded NPs were prepared at 1.5, 3.0 and 6.0% w/v overall polymer concentrations in the organic phase. Correspondingly, the formulations were named PPFC1.5, PPFC3 and PPFC6. Subsequently, acetone was evaporated overnight for NP hardening, and the resulting NP suspension was washed three times by centrifugation (Hettich Zentrifugen, Germany; 10,000 rpm, 30 min).

The NPs containing CD-CURC inclusion complex were obtained by preparing an internal aqueous phase (W0), composed of 640 μL of a CD-CURC solution in EtOH (1.4 mg/mL); in the case of placebo

Table 1
^{1}H chemical shifts of free HP-β-CD and in the presence of CURC.

Hydrogen	HP-β-CD	CD-CURC complex	Δδ[(a)]
H-1	4.8318	4.8329	+0.0011
H-2[(b)]	–	–	–
H-3	3.7484	3.7501	+0.0017
H-4[(b)]	–	–	–
H-5[(c)]	3.5637	3.5719	+0.0082
H-6	3.6194	3.6233	+0.0039
H-7[(b)]	3.3270	3.3270	–
H-8	5.0177	5.0207	+0.0030
H-9	1.0237	1.0244	+0.0007