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Research paper

Factorial design formulation optimization and *in vitro* characterization of curcumin-loaded PLGA nanoparticles for colon delivery

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ABSTRACT

We have employed a 2^3 factorial design approach for optimizing curcumin-loaded PLGA nanoparticles with a specific interest in colon treatment. Curcumin-loaded PLGA nanoparticles were prepared by using the single emulsion solvent evaporation technique. *In vitro* characterizations of the curcumin-loaded nanoparticles revealed that the mean particle sizes of the nanoparticles ranged from 181.5 nm to 206.9 nm, the zeta potential values were in the range of −30.6 to −41.7 mV, the encapsulation efficiencies were between 58.1 and 83.2% and the drug release from the formulations was in the range of 34.4–62.8%. The properties of the optimized curcumin-loaded PLGA nanoparticles predicted by the 2^3 factorial design approach correlated very well with the experimentally determined particle size of 219.6 nm, zeta potential of −36.8 mV, encapsulation efficiency of 74.4% and a 56.2% cumulative drug release after 24 h. *In vitro* cellular uptake studies with HT-29 cells showed that the optimized curcumin-loaded PLGA nanoparticle exhibited a much higher cellular uptake of curcumin (i.e. 7.01 ± 0.33 μg/10^6 cells) than a native curcumin solution (3.74 ± 0.56 μg/10^6 cells). Stability studies also showed that all investigated formulations were stable at least for 2 months.

1. Introduction

Colon cancer is the third leading cause of cancer deaths in the United States [1]. The American Cancer Society has estimated that there will be about 93 090 new cases of colon cancer and 39 610 new cases of rectal cancer in the United States in 2015, and these are expected to cause about 49 700 deaths during 2015. The lifetime risk of developing a colorectal cancer is about 1 in 20 [1]. The most common treatment for cancer is chemotherapy. However, chemotherapy is often associated with a number of drawbacks such as nonselective distribution of drugs, multidrug resistance, enhanced drug toxicity, undesirable side effects on normal tissue and inherent lack of beneficial response of cytotoxic anticancer drugs [2,3]. Therefore, there is an urgent need to develop therapeutic modalities with no or minimal side effects on healthy tissues or organs.

Natural herbal extracts, such as curcumin, hold high potential as chemotherapeutic agents because they are often safe and do not show side effects on healthy tissues or organs. They also show chemo preventive activities against malignancy. In recent years curcumin has drawn the attention of research because it sensitizes cancer cells for chemotherapy by inducing programmed cell death [4,5]. Curcumin is a polyphenol of turmeric derived from the roots (rhizomes) of Curcuma longa. Several studies have shown that curcumin has anti-inflammatory [6], antioxidant [7] and antimicrobial effects [8]. The most important effect is, however, its potential use against cancer due to its ability to suppress the proliferation of a wide variety of tumor cells [4,9]. Curcumin has been reported to be a potent inhibitor of the nuclear factor-kappa B (NF-kappa B) activation in various human cancer cell lines [10–12]. It has also been reported to decrease multidrug resistance by down regulating the P-glycoprotein (PGP) expression in resistant cells. Unfortunately, the high potential of curcumin for treatment of cancer and chronic inflammation is hampered by its low aqueous solubility at physiological pH conditions, rapid decomposition in alkaline media and instability towards light. For example, curcumin is practically insoluble in neutral aqueous solutions (i.e. ~ 1 μM), but

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dissolves readily in organic solvents such as alcohols, ketones, esters, and organic acids (i.e. in the range between 1 and 30 mM) [13]. The solubility of curcumin in dichloromethane has also been reported to be at least 3 mM [14]. The hydrophobic character of curcumin results in pharmacokinetic restrictions such as low absorption, poor bioavailability by oral route, extensive metabolism and rapid elimination [15]. Numerous approaches have been made to ameliorate the bioavailability of curcumin. These include the use of adjuvants, which can block metabolic pathways of curcumin (for example Piperine, a known inhibitor of hepatic and intestinal glucuronidation) [15]. The main strategies used to overcome the physicochemical limitations of curcumin and to increase its bioavailability are based on loading the compound in nanocarriers, such as liposomes [16,17], polymeric micelles [18], phospholipid complexes [19] and polymeric nanoparticles [15,20]. Nanocarriers have been shown to increase the solubility, provide longer circulation times, enhance permeability, and increase resistance towards metabolic processes of curcumin. Some formulations have been designed to prolong the release of curcumin, while others have additional mechanisms to improve the cellular delivery or intracellular release.

Biodegradable polymeric nanoparticles (PNPs) have widely been developed as nanoscale drug delivery vehicles for cancer treatment due to their excellent endocytosis efficiency, passive tumor-targeting, high encapsulation efficiency, and ability to deliver a wide range of therapeutic agents [21–23]. Nanoencapsulation protects the molecules from premature degradation, improves their solubility, and promotes controlled drug release and drug targeting. Nanoparticles (NPs) can at best represent a low risk of toxicity and they can often enhance the drug efficacy, i.e. specificity, tolerability and therapeutic index [24]. Poly (lactic-co-glycolic acid) (PLGA) is an approved biodegradable polymer with good biocompatibility. Therefore, PLGA and its various derivatives have widely been explored as carriers for controlled delivery of a large number of both small molecular drugs and macromolecular therapeutics [25,26]. The majority of PLGA-based drug delivery carriers are in the form of injectables, but PLGA-based nanoparticles are also continuously investigated for oral delivery [27–29].

The main objective of this study was to preliminary investigate the use of PLGA for encapsulating curcumin into PNPs for oral colon delivery purposes. A prerequisite for this is to confirm that curcumin-loaded PLGA nanoparticles withstand the harsh conditions in the stomach and small intestine before they reach the colon. Therefore, we have focused on the systematic optimization of the basic composition of curcumin-loaded PLGA nanoparticles with the aim to improve the aqueous dispersability of curcumin, to protect it from degradation in simulated gastrointestinal fluids and to increase curcumin uptake in colon cancer cells. Curcumin-loaded PNPs (C-PNPs) were prepared with an emulsion solvent evaporation technique by using a 2^3 factorial design approach to optimize the composition of the C-PNP. The physicochemical properties and *in vitro* characterization of C-PNPs were determined and performed through particle size, zeta potential, drug encapsulation, drug release kinetics, Fourier-Transform infrared spectroscopy and differential scanning calorimetry studies. Furthermore, the stability of the C-PNPs at room temperature and 4 °C, the stability of the optimized C-PNP against enzyme and bile salt, and the *in vitro* cellular uptake of the optimized C-PNP formulation in colon cancer cells were also investigated.

2. Materials and methods

2.1. Materials

Curcumin was purchased from Cayman chemical company (China). Acid terminated Poly (D, L-lactide-co-glycolide) (PLGA, 50:50, Mw = 7.000–17.000, Resomer® RG 502H), Polyvinyl alcohol (PVA, mw 31,000–50,000 – 87–89% hydrolyzed), Dichloromethane (DCM) and Phosphate Buffer Saline (PBS) tablet were purchased from Sigma–Aldrich (Germany). Potassium dihydrogen phosphate was purchased from Riedel-de-Haen (Germany). Acetonitrile, Phosphoric acid and Methanol were purchased from Fluka (United Kingdom). OPTI-MEM I, McCoy's 5a Medium, and TrypLE Express were obtained from Gibco, Life Technologies (USA).

2.2. Preparation and characterization of the formulations

2.2.1. Experimental design

Statgraphics® plus software version 4 was used to adapt the 2^3 factorial design approach to optimize the curcumin-loaded PLGA nanoparticle (C-PNP). The independent variables for optimization were 1) the amount of PLGA in the organic phase (X_1 = c PLGA), 2) the PVA concentration in the aqueous phase (X_2 = c PVA) and 3) the amount of curcumin in the organic phase (X_3 = c CUR). The selected responses were 1) the mean particle size of the C-PNPs (Y_1), 2) the zeta potential of the C-PNPs (Y_2), 3) the encapsulation efficiency (Y_3) and 4) the percentage (%) of the cumulative drug released after 24 h (Y_4). Each independent variable was given a high and low level value as shown in Table 1.

2.2.2. Preparation of curcumin-loaded PLGA nanoparticles (C-PNPs)

C-PNPs were prepared by using the single emulsion-solvent evaporation technique reported by Khalil et al. [24] with slight modifications. Briefly, 100–200 mg of PLGA polymer was dissolved in 5 ml of dichloromethane (DCM) in a glass tube. Then 10 or 20 mg of curcumin powder was added to the polymer/solvent mixture and allowed to dissolve for 30 min, with intermittent vortexing. The organic phase containing PLGA and curcumin was rapidly added drop wise into a glass tube containing 10 ml of PVA in an aqueous solution, while vortexing. Once all the drug/polymer mixture was added to the PVA solution, the contents were vortexed for an additional 10 s at a high setting. The tube contents were then emulsified by using sonication for 7 min at 40% amplitude in an ice - water bath by using a probe sonicator (Vibra-Cell VCX 750 sonicator, Sonics & Materials Inc., Newtown, CT, USA). The resulting fine (O/W) emulsion was immediately poured into 30 ml of an aqueous PVA 0.5% w/v solution under rapid stirring with a magnetic stirrer. Dichloromethane was then evaporated from the droplets under magnetic stirring at 800 rpm for 3 h. The nanoparticles were collected by centrifugation at 20,000 rpm for 15 min and washed 3 times with Milli-Q water. Supernatants were collected to evaluate the encapsulation efficiency of curcumin. Finally, the pellet of the nanoparticles was re-suspended in 5 ml of Milli-Q water.

2.2.3. Physicochemical characterization and drug encapsulation efficiency

2.2.3.1. Particle size determination. The average particle size and PDI of the C-PNPs were measured with dynamic light scattering by using a ZetaSizer Nano ZS instrument (Malvern Instruments Ltd,

Table 1
Independent and dependent variables used for the 2^3 factorial design approach.

Independent variables	Level		Dependent variables
	Low	High	
Amount of PLGA	100 mg	200 mg	Particle size
Concentration of PVA	2%	4%	Zeta potential
Amount of curcumin	10 mg	20 mg	Encapsulation efficiency
			In vitro release over 24 h.