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Research paper

Stable curcumin-loaded polymeric micellar formulation for enhancing cellular uptake and cytotoxicity to FLT3 overexpressing EoL-1 leukemic cells

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ABSTRACT

The present study aims to develop a stable polymeric micellar formulation of curcumin (CM) with improved solubility and stability, and that is suitable for clinical applications in leukemia patients. CM-loaded polymeric micelles (CM-micelles) were prepared using poloxamers. The chemical structure of the polymers influenced micellar properties. The best formulation of CM-micelles, namely CM-P407, was obtained from poloxamer 407 at drug to polymer ratio of 1:30 and rehydrated with phosphate buffer solution pH 7.4. CM-P407 exhibited the smallest size of 30.3 ± 1.3 nm and highest entrapment efficiency of 88.4 ± 4.1%. When stored at −80 °C for 60 days, CM-P407 retained high protection of CM and had no significant size change. In comparison with CM solution in dimethyl sulfoxide (CM-DMSO), CM kinetic degradation in both formulations followed a pseudo-first-order reaction, but the half-life of CM in CM-P407 was approx. 200 times longer than in CM-DMSO. Regarding the activity against FLT3 overexpressing EoL-1 leukemic cells, CM-P407 showed higher cytotoxicity than CM-DMSO. Moreover, intracellular uptake to leukemic cells of CM-P407 was 2–3 times greater than that of CM-DMSO. These promising results for CM-P407 will be further investigated in rodents and in clinical studies for leukemia treatment.

1. Introduction

Many dietary natural products have been recently evaluated as potential therapeutic agents for a wide range of diseases [1,2]. Curcumin (CM), a yellow pigmented substance, is a major active curcuminoid in rhizomes of turmeric (*Curcuma longa* L.). Two other active curcuminoids often included in the commercial CM are demethoxycurcumin and bisdemethoxycurcumin. CM in the form of a crude powder of turmeric rhizome has been employed as an alternative traditional medicine during the past few decades with little or no apparent toxicity [3]. Previous studies reported that CM exhibited anti-oxidant, anti-inflammatory, and anticancer activities against various types of cancer cells [4,5]. For leukemia, CM has shown an excellent cytotoxicity to both standard cell lines and clinical leukemic cells isolated from patients [6,7]. Moreover, our recent study reported that CM inhibited cell growth and FLT3 protein level in FLT3 overexpressing EoL-1 leukemic cells [8]. Overexpression of FLT3 in leukemic cells is related to the increase in survival and proliferation rate of leukemic blast cells [9,10]. Although CM expressed high inhibitory activity against leukemic cells, all experiments mentioned above were performed *in vitro* where CM was present in an organic solvent such as dimethyl sulfoxide (DMSO) because of the low aqueous solubility of CM. This limits clinical applications, as DMSO is toxic to humans. Moreover, the short circulation time [11] and rapid metabolism [12] of CM decrease its half-life, thus limiting the persistence of CM in the body and hindering its therapeutic effect. To enhance the bioavailability of CM, various techniques of fabricating suitable CM formulations have been studied [13]. Nanodelivery systems are currently popular approaches for improving drug dissolution and for carrying hydrophobic drugs to target tissues [14,15]. Many types of nanodelivery systems, such as liposomes and nanoemulsions have been developed to overcome the poor bioavailability of CM [16,17]. However, liposomes have disadvantages on stemming

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from rapid degradation along the oxidation and hydrolysis pathways [18]. The high amount of surfactant used in nanoemulsions can also lead to unwanted side effects [19]. Polymeric micelles is one attractive nanodelivery system used to improve the bioavailability of poorly soluble drugs; this is due to the simplicity of fabrication and high stability of the entrapped drugs [20].

Various types of polymers have been used to formulate polymeric micelles. Poloxamers are a group of polymers that have been proven to be safe and effective nanocarriers for hydrophobic drugs [21]. These tri-block copolymers consist of 2 hydrophilic chains of poly-ethylene oxide (PEO) and 1 hydrophobic chain of poly-propylene oxide (PPO) with different numbers of PEO and PPO units. As a result of their amphiphilic character, the PEO-PPO-PEO block copolymers act like a surfactant molecule that can interact with hydrophobic surfaces and biological membranes. Moreover, the self-assembly of these copolymers into micelles can be achieved in aqueous solution above their critical micellar concentrations [22]. These characteristics improve the aqueous solubility of hydrophobic drugs [23]. Many previous studies suggest that poloxamers are the polymer of choice for formulating poorly soluble drugs in order to improve their stability and facilitate delivery [24,25]. Many research groups have investigated poloxamer based CM-loaded nanoparticles for improvement of solubility and bioavailability of CM [26,27]. However, there are many factors affecting the success of fabrication; these include the type of polymer, polymer to CM ratio, methods and procedures, type of reagent and diluent. On the other hand, the particle size, entrapment efficiency, stability, and release property are also important aspects of production. Suitable polymeric micelle size should be smaller than 100 nm [28]. Previous loading of CM in nanocarriers was reported, but the size is too big and wide size distribution led to unsuccessful targeting [29]. The large size and wide size distribution of the obtained micelles might have been due to the concentration or unsuitable ratio of both polymers. Some CM-loaded micelles needed more than 1 h for fabrication, and about 24 h for purification or elimination of the organic solvent [30,31]. Some of the organic solvents used in the formulation have high boiling points, and thus need high temperatures and long periods to completely eliminate the solvent. This in turn might affect the activity of heat labile compounds such as CM.

Leukemia is one of the most common hematological malignancies, occurring with a high incidence and mortality rate worldwide. To the best of our knowledge, research focused on using CM-loaded polymeric micelles for anti-leukemia purpose is very rare. The overexpression of leukemia-related genes and their proteins is a useful starting point for leukemia treatment. Feline McDonough Sarcoma (FMS)-like tyrosine kinase 3 (FLT3), is a leukemia-related protein and leukemia marker that overexpresses on the cell surface of leukemic cells, especially acute myelogenous leukemia (AML) and B lineage acute leukemia. The present study thus aims to improve the aqueous solubility and stability of CM suitable for clinical use in FLT3 overexpressing leukemia patients. A highly stable polymeric micellar formulation of CM with high cellular uptake and cytotoxicity to FLT3 overexpressing EoL-1 leukemic cells was developed. Two common polymers, poloxamer 188 (P188) and poloxamer 407 (P407), were used to fabricate micelles. CM-loaded polymeric micelles (CM-micelles) were prepared using a simple method that can be easily operated on a large scale. Formulations were optimized using variables that would yield small size and stable CM-micelles with high drug entrapment efficiency (EE). Various types of hydrating agent, diluent, and concentrations of bovine serum albumin (BSA) were used to evaluate their effects on the properties of CM-micelles. The most optimal formulation was selected for investigation on physical stability of CM-micelles and chemical stability of CM as well as *in vitro* release property of the micelles. Finally, the ability of the selected CM-micelle formulation to deliver CM to FLT3 overexpressing EoL-1 leukemic cells and their cytotoxic effect on the leukemic cells were investigated.

2. Materials and methods

2.1. Materials

CM used was a commercial grade compound of turmeric curcuminoids (Product No. C1386) obtained from Sigma-Aldrich (St Louis, MO). This is a mixture of 80% CM, 15% demethoxycurcumin, and 5% bisdemethoxycurcumin. P407 and P188 were purchased from Sigma-Aldrich. Dichloromethane (CH_2Cl_2), BSA, fetal bovine serum (FBS), dialysis bags, DMSO, and phosphate buffer solution (PBS) were obtained from Fisher Scientific (Pittsburgh, PA). The 1640 Roswell Park Memorial Institute (RPMI-1640) (with and without phenol red) were purchased from Life Technology (Carlsbad, CA). Triton X-100 was purchased from Atlanta Biological (Flowery Brance, GA). Penicillin/Streptomycin was purchased from MP Biomedicals (Santa Ana, CA). TEM carbon film copper grid and paraformaldehyde were purchased from Electron Microscopy Sciences (Hatfield, PA). A hermetic aluminum sample pan was purchased from DSC Consumables Inc. (Austin, MN). The 96-well glass bottom plate was purchased from *In Vitro* Scientific (Sunnyvale, CA).

2.2. Preparation of CM-micelles

Exact volumes of 1.000, 0.500, 0.333, 0.250, 0.200, 0.167, 0.143, 0.125, 0.111, and 0.100 mL of stock CM were mixed with 1.000 mL of stock polymer solution to obtain serial working solutions containing CM and polymer at weight ratios of 1:10, 1:20, 1:30, 1:40, 1:50, 1:60, 1:70, 1:80, 1:90, and 1:100, respectively. Total amount of organic solvent in each system was 2.0 to 1.1 mL. The mixture was transferred to a 50-mL round bottom flask (RBF). The solvent was evaporated using rotary evaporation at 40 °C and a RBF rotation speed of 100 rpm for 10 min to obtain a CM-polymer film. The residual CH_2Cl_2 remaining in the film was removed by air drying in a fume hood at room temperature overnight. The films were rehydrated in 4 mL of deionized distilled water (ddH_2O) or PBS by extensive mixing to obtain CM-micelles. Non-encapsulated CM was separated by centrifugation at 5000 rpm for 5 min and filtration through a 0.22 μm membrane. Blank micelles were prepared according to the same procedure without CM.

2.3. Investigation of physical properties of CM-micelles

Physical properties of CM-micelles were evaluated. The size and size distribution (PdI) as well as zeta potential (ZP) of CM-micelles were characterized and compared with those of the blank micelles using photon correlation spectroscopy (PCS; Zetasizer, Malvern Instrument, UK) and ZetaPALS Particle Sizing (Brookhaven Instrument Corp, NY). Determination of CM in the micelles was done using UV–visible spectrophotometer (Agilent 8453, Agilent Technologies, CA) at 425 nm and calculated using the standard curve of CM in methanol [32]. Entrapment efficiency (EE) and loading capacity (LC) of the micelles were calculated according to Eqs. (1) and (2), respectively.

$$EE\ (\%) = 100C_m/C_t \quad (1)$$

$$LC\ (\%) = 100C_m/P_t \quad (2)$$

where C_m is the amount of CM in a CM-micelle, C_t is the amount of total CM used, and P_t is the amount of total polymer used.