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Curcumin loaded polymeric micelles inhibit breast tumor growth and spontaneous pulmonary metastasis

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ABSTRACT

This work aims to develop curcumin (Cur) loaded biodegradable self-assembled polymeric micelles (Cur-M) to overcome poor water solubility of Cur and to meet the requirement of intravenous administration. Cur-M were prepared by solid dispersion method, which was simple and easy to scale up. Cur-M had a small particle size of 28.2 ± 1.8 nm and polydisperse index (PDI) of 0.136 ± 0.050, and drug loading and encapsulation efficiency of Cur-M were $14.84 \pm 0.11\%$ and $98.91 \pm 0.70\%$, respectively. Besides, *in vitro* release profile showed a significant difference between rapid release of free Cur and much slower and sustained release of Cur-M. Cytotoxicity study showed that the encapsulated Cur remained its potent anti-tumor effect. Furthermore, Cur-M were more effective in inhibiting tumor growth and spontaneous pulmonary metastasis in subcutaneous 4T1 breast tumor model, and prolonged survival of tumor-bearing mice. In addition, immunofluorescent and immunohistochemical studies also showed that tumors of Cur-M-treated mice had more apoptosis cells, fewer microvessels, and fewer proliferation-positive cells. In conclusion, polymeric micelles encapsulating Cur were developed with enhanced anti-tumor and anti-metastasis activity on breast tumor, and Cur-M is excellent water-based formulation of Cur which may serve as a candidate for breast cancer therapy.

1. Introduction

As a major public health problem, cancer gains increasing incidences and mortalities every year in the world. Breast cancer is the most common and the second lethal malignancy in women in United States (Siegel et al., 2012). Surgery is an effective method for patients with breast cancer. However, recurrence and metastasis often ensues after surgery, which would compromise the therapeutic effect. Chemotherapy was employed to treat or prevent the recurrence and metastasis after surgery, which could prolong the survival of cancer patients (Gong et al., 2012c). However, chemotherapy also showed severe side effects, such as immune suppression and myelosuppression, which dramatically limited the intensity of chemotherapy and declined the life quality of patients (Armstrong et al., 2006). Besides, widespread distribution and rapid elimination of chemotherapeutic drugs required large dosage to keep their therapeutic concentration, which also enhanced their side effects (Gong et al., 2012a). Therefore, novel drug delivery systems (DDSs) are desirable to improve the drug concentration at tumor site and reduce the side effects (Allen and Cullis, 2004).

Curcumin (Cur) is a nature polyphenolic phytoconstituent known as diferuloylmethane (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) (Singh, 2007). Cur is the active component of turmeric, which has been widely used in Southeast Asia countries as spice or traditional medicine (Jagetia and Aggarwal, 2007). In previous reports, Cur showed anti-inflammatory, anti-oxidant, anti-bacteria, anti-virus, anti-tumor, and hyperlipidemic activities (Maheshwari et al., 2006; Tang et al., 2010; Yallapu et al., 2012). However, owing to poor solubility (Safavy et al., 2007), poor oral bioavailability, and extensive fist pass metabolism (Anand et al., 2007), the therapeutic efficacy of Cur was limited. Therefore, a water-based formulation with controlled release property is needed for clinical application of Cur (Li et al., 2005).

Water solubility of drugs is a critical factor in the drug development (Gong et al., 2010). Approximately half of new reactive molecular entities were not successfully developed because of their hydrophobicity (Gong et al., 2012b). Nanotechnology, as a fast developing field, provides an important method to overcome the problems of hydrophobic drugs (Allen and Cullis, 2004; Chawla and Amiji, 2002; Farokhzad et al., 2006; Hennenfent and Govindan, 2006; Wagner et al., 2006). Polymeric micelles are

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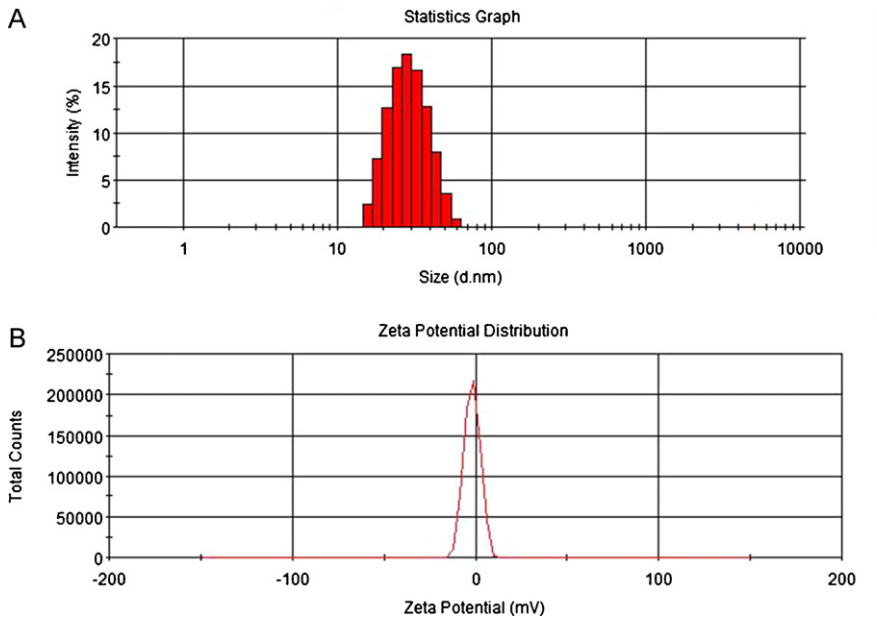

Fig. 1. Particle size (A) and zeta potential (B) of prepared Cur-M.

widely used as drug delivery system to improve the water solubility and anti-tumor activity of hydrophobic chemotherapeutic agents (Oerlemans et al., 2010). After hydrophobic agents are rendered to form nano-scale micelles, they can be well dispersed in aqueous solution to form a stable and homogeneous solution (suspension), therefore meet the requirements of clinical intravenous administration (Gong et al., 2011). Furthermore, *in vivo* circulation time of micelles is prolonged due to their nano-size and present of hydrophilic shell. Besides, owing to the enhanced permeability and retention (EPR) effect, micelles can passively target to tumor site, which can improve their anti-tumor effects (Fang et al., 2011).

In our previous work, Cur loaded nanoparticles were prepared by nano-precipitation method and investigated for their therapeutic effect on subcutaneous colon tumor model (Gou et al., 2011). In this work, a one-step solid dispersion method was employed to prepare Cur loaded polymeric micelles (Cur-M) with small particle size and high drug loading, and the characteristics of prepared Cur-M were investigated as a potential water-based formulation of Cur. Besides, *in vitro* release behaviors of Cur-M and free Cur were investigated using a modified dialysis method. Cytotoxicity evaluation of Cur-M and free Cur was also conducted. Furthermore, anti-tumor and anti-metastasis activity of Cur-M were evaluated in a subcutaneous 4T1 breast tumor model using free Cur, blank micelles, and normal saline (NS) as control groups. Tumor growth and spontaneous pulmonary metastasis of subcutaneous 4T1 breast tumor model were evaluated in detail.

2. Materials and methods

2.1. Materials

Monomethyl poly(ethylene glycol) (MPEG, Mn = 2000, Fluka, USA), ε-caprolactone (ε-CL, Alfa Aesar, USA), stannous octoate ($Sn(Oct)_2$, Sigma, USA), curcumin (Sigma, USA), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (methyl thiazolyl tetrazolium, MTT, Sigma, USA), Roswell Park Memorial Institute 1640 medium (RPMI 1640, Gibco, USA), and methanol (HPLC grade, Fisher Scientific, UK), were used without further purification. All the materials used in this article were analytic reagent (AR) grade and used as received.

Monomethyl poly(ethylene glycol)-poly(ε-caprolactone) copolymer (MPEG-PCL) were synthesized according to our previous work (Gong et al., 2009, 2012b), and molecular weight of MPEG-PCL is 3950 (determined by ^{1}H NMR).

4T1 cells and L929 cells were purchased from the American Type Culture Collection (ATCC; Rockville, MD), which grew in RPMI 1640 supplement with 10% fetal bovine serum (FBS). The cells were maintained in a 37 °C incubator with a humidified 5% CO_2 atmosphere.

BALB/c mice (6–8 weeks) were used for *in vivo* anti-tumor tests, which were purchased from the Laboratory Animal Center of Sichuan University. Animals were housed at controlled temperature of 20–22 °C, relative humidity of 50–60% and 12 h light–dark

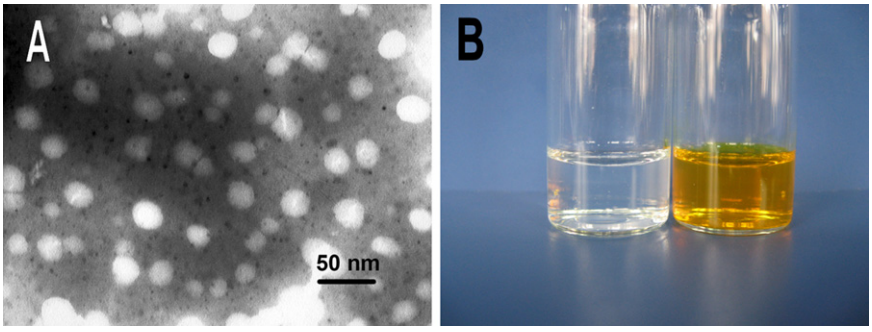

Fig. 2. Morphology of Cur-M. (A) TEM image of Cur-M; (B) appearance of blank micelles (left) and Cur-M (right).