

RESEARCH ARTICLE

In Vivo Evaluation of Curcumin-loaded Nanoparticles in a A549 Xenograft Mice Model

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Abstract

Curcumin (Cum) has been reported to have potential chemo-preventive and chemotherapeutic activity through influencing various processes, inducing cell cycle arrest, differentiation and apoptosis in a series of cancers. However, the poor solubility of Cum limits its further applications in the treatment of cancer. We have previously reported Cum-loaded nanoparticles (Cum-NPs) prepared with amphiphilic methoxy poly(ethylene glycol)-polycaprolactone (mPEG-PCL) block copolymers. The current study demonstrated superior antitumor efficacy of Cum-NPs over free Cum in the treatment of lung cancer. *In vivo* evaluation further demonstrated superior anticancer effects of Cum-NPs by delaying tumor growth compared to free Cum in an established A549 transplanted mice model. Moreover, Cum-NPs showed little toxicity to normal tissues including bone marrow, liver and kidney at a therapeutic dose. These results suggest that Cum-NPs are effective to inhibit the growth of human lung cancer with little toxicity to normal tissues, and could provide a clinically useful therapeutic regimen. They thus merit more research to evaluate the feasibility of clinical application.

Keywords: Curcumin - nanoparticle - in vivo - lung cancer - antitumor efficacy

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Introduction

Lung cancer is a disease with poor overall survival. 5-year survival rate of patients with lung cancer of all stages is only 16% (Jemal et al., 2009; Rocks et al., 2012). Therefore, it is important to identify potential regimens and explore more efficient therapeutic strategies for the treatment of lung cancer.

Curcumin (Cum), the principal polyphenolic curcuminoid, obtained from the turmeric rhizome *Curcuma longa*, has been reported for its potential chemo-preventive and chemotherapeutic activity by influencing different stages of carcinogenesis, including cell cycle arrest, differentiation, and apoptosis in a series of cancers (Pan et al., 2000; Surh et al., 2001; Aggarwal et al., 2003; Duvoix et al., 2005; Aggarwal et al., 2009; El-Azab et al., 2011). For example, *In vitro* and *in vivo* experiments show the ability of Cum in inhibiting skin squamous cell carcinoma growth and blocking tumor progression (Phillips et al., 2011).

In our previous research, we prepared Curcumin-loaded methoxy poly(ethylene glycol)-polycaprolactone (mPEG-PCL) nanoparticles (Cum-NPs) (Yin et al., *in press*). The drug loaded content of Cum-NPs is more than 15% and the encapsulation efficiency is around 85%. The

in vitro release test demonstrated the sustained release pattern of Cum-NPs at room temperature. Moreover, *in vitro* cytotoxicity test showed that Cum-NPs inhibited the growth of A549 cells in a time and dose dependent manner. Apoptotic staining demonstrated the superior pro-apoptotic effect of Cum-NPs over the free drug.

In the current study, we hypothesize that Cum-NPs could have *in vivo* anticancer efficiency in a xenograft model of A549 cells. The growth curve of tumor volume and bodyweight of the mice will be measured every other day. At the end of *in vivo* experiment, mice will be sacrificed to detect the influence of Cum-NPs on the peripheral blood parameters and liver and kidney functions.

Materials and Methods

Materials

Curcumin, was purchased from Sigma Chem. Co., (St. Louis, USA). PEG samples were dehydrated by azeotropic distillation with toluene, and then vacuum dried at 50 °C for 12 h before use. ε-CL was purified by drying over CaH₂ at room temperature and distillation under reduced pressure. Stannous octoate (Sigma) were used as received. All other chemicals were of analytical grade and used

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without further purification. Human lung cancer cell line A549 was obtained from Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China).

Male and female nude mice (nu/nu; 6–8 weeks old and weighing 18–22 g) were purchased from Model Animal Research Center of Nanjing University (Nanjing, China). The mice were housed and maintained in the animal facility of the Animal Center of Nanjing Medical University. The animal protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Nanjing Medical University.

Preparation and characterization of Cum-loaded mPEG-PCL nanoparticles

Cum-NPs were prepared by a nano-precipitation method as described previously with minor modification (Yin et al., in press). Briefly, 10 mg of each copolymer and 2 mg Cum were dissolved in 0.3ml hot acetone. The obtained organic solution was added dropwise into 10 times volumes of distilled water under gentle stirring at room temperature. The solution was dialyzed in a dialysis bag (molecular weight cut-off 4kd, Sigma) to remove acetone thoroughly. The resulted bluish aqueous solution was filtered through a 0.22 μm filter membrane to remove non-incorporated drugs and copolymer aggregates. The prepared nanoparticles were lyophilized for further use.

In vivo antitumor efficacy

Nude mice implanted with A549 cell line were used to qualify the antitumor efficacy of Cum and Doc, alone or in combination, through intravenous administration. The mice were raised under specific pathogen-free (SPF) circumstances and all of the animal experiments were performed in full compliance with guidelines approved by the Animal Care Committee of Nanjing Medical University. The mice were subcutaneously injected at the left axillary space with 0.1 ml of cell suspension containing $4\text{--}6 \times 10^6$ A549 cells. Treatments were started after 7–8 days of implantation. The mice whose tumor reached a tumor volume of 100 mm^3 were selected and this day was designated as “Day 0”.

On Day 0, the mice were randomly divided into four groups, with each group being composed of 6 mice. The mice were treated intravenously with saline, blank NPs, free Cum and Cum-NPs, respectively. Cum was administered at a equivalent dose of 15 mg/kg. All mice were tagged, and tumors were measured every other day with calipers during the period of study. The tumor volume was calculated by the formula $(W^2 \times L)/2$, where W is the tumor measurement at the widest point, and L is the tumor dimension at the longest point.

Each animal was weighed at the time of treatment so that dosages could be adjusted to achieve the mg/kg amounts reported. Animals also were weighed every other day throughout the experiments. After 15 days of injections, the mice were sacrificed for the detection of peripheral blood parameters as well as liver and kidney functions.

Statistical analysis and research experience

Results were presented as Mean \pm SD. Statistical

comparisons were made by t test or ANOVA analysis. The accepted level of significance was P value < 0.05 . We have enough experience in conducting medical researches, and have published some results elsewhere (Huang et al., 2004; Zhou et al., 2009; Jiang et al., 2010; Yan et al., 2010; Gao et al., 2011; Huang et al., 2011; Li et al., 2011; Li et al., 2011; Li et al., 2011; Xu et al., 2011; Xu et al., 2011; Xu et al., 2011; Yan et al., 2011; Zhang et al., 2011; Gong et al., 2012; Li et al., 2012; Yu et al., 2012).

Results

In vivo antitumor evaluation of Cum-NPs against A549 xenograft

Antitumor efficacy of Cum-NPs was investigated in A549 human lung cancer xenografts in nude mice. It is observed from Figure 1A that blank NPs showed no tumor growth inhibition effect compared to control group, while both Cum and Cum-NPs significantly inhibited the growth of lung cancer since Day 4 ($P < 0.05$ vs control). Moreover, delivery of Cum in nanoparticles inhibited the growth of tumor more efficiently than free Cum ($P < 0.05$). Among the four groups, the group that received Cum-NPs was observed to maintain the greatest amount of antitumor activity (Figure 1A&C). At the end of treatment, The RTV of the group received free Cum is 10.42 ± 1.23 . The RTV of the group received Cum-NPs is 6.24 ± 0.59 , which is the lowest among all the groups indicating the strongest tumor inhibition. Statistical analysis reveals the significant differences between the group receiving Cum-NPs and the group receiving free Cum. Figure 1C showed the

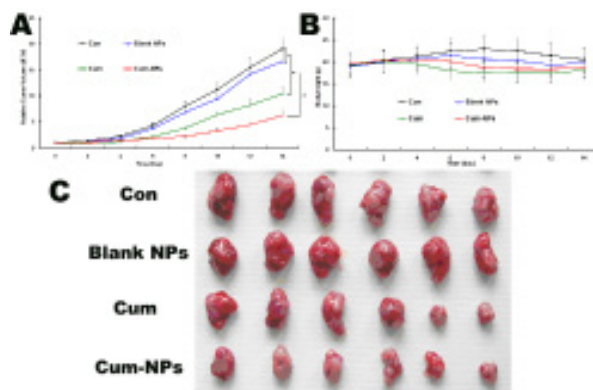


Figure 1. (A) Tumor volume of established A549 xenografts in nude mice during therapy under different treatments. Mice were treated with different protocols on Day 0 (arrow) as showed in the figure. Saline: vehicle; blank NPs: empty nanoparticles; Cum: free Cum at a dose of 15 mg/kg; Cum-NPs: Cum-loaded nanoparticles in a saline solution at equivalent Cum doses of 15 mg/kg. Different agents were delivered through intravenous pathway when tumor volume measured 100 mm^3 . Data are presented as mean \pm SD (n = 6). The difference between tumor volumes in the group of saline and Cum or Cum-NPs is significant ($P < 0.05$). Significant difference ($P < 0.05$) also is observed between the group of free Cum and Cum-NPs at the equivalent dose. *represents $P < 0.05$. (B) Bodyweight change of nude mice receiving different treatments during therapy. Data are presented as mean \pm SD (n = 6). (C) The images of excised tumors at the time of sacrifice from the subcutaneous A549 lung adenocarcinoma xenograft-bearing male nude mice after 14 days of single dose therapy

Table 1. Influence of Cum-NPs on Peripheral Blood Parameters

Groups	WBC (10 ⁹ /L)	Hb (g/L)	Plt (10 ⁹ /L)
Con	22.3±2.3	109.3±11.2	140.2±17.4
Blank NPs	23.4±1.7	110.2±13.2	138.2±13.1
Cum	20.3±3.5	107.2±9.3	140.2±15.1
Cum-NPs	23.2±2.3	112.5±12.5	139.1±11.2

Table 2. Influence of Cum-NPs on Liver and Kidney Parameters

Groups	ALT (U/L)	BUN	Cre
Con	37.4±7.3	6.7±1.3	43.4±7.2
Blank NPs	41.4±7.5	6.9±1.1	47.1±6.6
Cum	42.3±16.4	7.3±1.2	39.6±8.2
Cum-NPs	39.3±8.6	7.8±1.6	43.9±6.2

shrinkage of tumors during the treatment groups. It could be observed clearly that the tumors taken from the mice receiving Cum-NPs were obviously smaller than those of other groups.

An analysis of body weight variations generally defined the adverse effects of the different therapy regiments (Figure 1B). No significance was observed among the four groups. However, the mice receiving free Cum were in a weak state in the aspects of movement and spirit, whereas no obvious alteration was observed in the Cum-NPs treated animals.

Influence of Cum-NPs on peripheral blood parameters

Table 1 indicates the influence of different agents on the peripheral blood parameters of the mice. No adverse effect was observed in mice treated by Blank NPs. As expected, Cum-NPs and Cum showed no severe influence on WBC and Hb. Neither did each of the agents damaged the liver and kidney function in the experimental time (Table 2).

Discussion

Here we reported that a spherical coreshell structure Cum-NPs formed by amphiphilic mPEG-PCL block copolymers demonstrated superior antitumor efficacy against lung cancer in vivo. In the current research, a xenograft model of human lung cancer was established in nude mice to evaluate the efficiency and toxicity of Cum-NPs. Previous study from the author's lab proved the dose dependent growth inhibition effect of Cum-NPs against A549 cells (Yin et al., in press). The present study further evaluated the in vivo antitumor efficacy of Cum-NPs in A549 xenografts. Cum-NPs significantly delayed tumor growth when compared with free Cum. In addition, Cum-NPs showed no adverse influence on peripheral blood parameters and organ functions.

The targeted release of Cum-NPs together with chemotherapy drugs will be paid more attention to further expand the application of this current research. For example, co-delivery of Cum and Paclitaxel would be of great significance in future in that the synergistic antitumor effect of Cum and Paclitaxel has been already demonstrated in previous studies (Ganta et al., 2009; Hossain et al., 2012). Moreover, increasing the targeting

ability of Cum-NPs by receptor-targeting peptides remains to be the most attractive research focus (Rothdiener et al., 2010; Franzen et al., 2011; Guo et al., 2011). It is hypothesized that a combination of passive targeting, with receptor targeting peptides, may significantly amplify the antitumor activity of the drug delivery system (Yu et al., 2010; Cutler et al., 2013; Liu et al., 2013). Due to the molecular recognition of peptides by tumor cell surface receptors, site-specific drug uptake by tumor cells may be raised, which may then lead to enhanced cytotoxicity (Hosta-Rigan et al., 2010; Almansour et al., 2012).

The current research reported the satisfied antitumor efficiency of a spherical core-shell structure Cum-NPs formed by amphiphilic mPEG-PCL block copolymers. In vivo evaluation further demonstrated the superior anticancer efficacy of Cum-NPs compared to free Cum in an established A549 transplanted mice model. Moreover, Cum-NPs showed little toxicity to normal tissues including bone marrow, liver and kidney at its therapeutic dose. It is concluded that nano formulation of Cum delivery is a most promising way in countering the spread of lung cancer, and continuing research will definitely advance the current study.

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