Facile synthesis of cetyltrimethylammonium bromide-loaded mesoporous silica nanoparticles for efficient inhibition of hepatocellular carcinoma cell proliferation

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Abstract

We report the synthesis of cetyltrimethylammonium bromide (CTAB)-loaded mesoporous silica nanoparticles (MSNs) as efficient anticancer agents. The chemical composition and structure, drug loading capacity, and in vitro cytotoxicity of CTAB-MSNs were analyzed. Our results reveal that CTAB-MSNs display a uniform pore structure, extensive surface area, and high drug-loading capacity, of approximately 37.2% by weight. The viability assessments showed that CTAB-MSNs exhibited superior human hepatoma (HepG2) cell killing compared to free CTAB. Therefore, CTAB-MSNs may have advantages over conventional cancer therapies.

1. Introduction

Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related deaths worldwide, and its incidence is increasing in many developed countries. The use of cytotoxic drugs is currently an effective method for treating solid tumors, such as liver and lung cancers [1]. However, HCC is highly resistant to anticancer drugs [2, 3]. Drug candidates for solid tumors are still evaluated primarily according to their ability to induce tumor shrinkage [4], and there remains a strong clinical need for well-established anticancer chemotherapeutic approaches.

Cetyltrimethylammonium bromide (CTAB) is a cationic surfactant that has been widely used as a broad-spectrum antibacterial agent [5–9]. Recently, several groups demonstrated that the potent anticancer activity of CTAB occurs via induction of mitochondrial apoptosis in cancer cells, by activating AMP-activated protein kinase and p53 signaling pathways [10–12]. Compared with conventional anticancer drugs, including cisplatin (CDDP), hydroxyamptothecin (HCPT), methotrexate (MTX), and doxorubicin (DOX), CTAB molecules are less expensive, more stable, and easier to use. These attributes make CTAB a potential therapeutic candidate for cancer treatment.

Mesoporous silica nanoparticles (MSNs) have attracted much attention because of their potential applications in sensing, catalysis, and drug delivery [13–18]. MSNs are also used as carriers in controlled drug delivery systems, owing to their excellent biocompatibility, high specific surface area and pore volume, and tunable pore structure.

Herein, we report the synthesis of CTAB-loaded mesoporous silica nanoparticles (CTAB-MSNs) for use as efficient anticancer materials. The chemical composition and structure, drug loading capacity, and in vitro cytotoxicity of CTAB-MSNs were evaluated. Our results have demonstrated that nanomaterials MSNs displayed lower cytotoxicity towards human hepatoma (HepG2) cells. Moreover, MSNs loaded with CTABs inhibit hepatocarcinoma cell growth better than free CTABs.

2. Materials and methods

2.1. Materials

Tetraethoxysilane (TEOS) was purchased from Alfa Aesar (Haverhill, MA, USA). CTAB, sodium hydroxide (NaOH), and ammonium nitrate (NH₄NO₃) were obtained from Aladdin (Shanghai, China). All other
chemicals were of analytical grade and were used without further purification. For *in vitro* experiments, Dulbecco’s modified Eagle’s medium/Ham’s F12, newborn calf serum, penicillin, and streptomycin were all purchased from Beyotime Biotechnology (Shanghai, China).

### 2.2. Preparation of CTAB-MSNs

A mixed solution was prepared by dissolving 170 mg CTAB and 50 mg NaOH in 80.0 ml deionized water, at 80 °C with constant stirring. TEOS (0.833 ml) was quickly added dropwise to the mixture and vigorously stirred for 2 h. A white precipitate was isolated by centrifugation, washed with pure water, and air-dried at 60 °C for 24 h.

### 2.3. Removal of CTAB molecules from CTAB-MSNs by ion exchange

CTAB-MSN (0.1 g) was dispersed in 30 ml of 95% ethanol containing 0.2 g of ammonium nitrate (NH₄NO₃). The mixture was stirred at 25 °C for 6 h, and the product was collected by centrifugation and washed with ethanol. The above treatment was repeated twice.

### 2.4. Cell proliferation activity assay

HepG2 cells were seeded in 24-well plates (5 × 10⁴ cells per well). After overnight culture, MSNs, free anticancer drug (CTAB), and CTAB-MSNs were added at different concentrations. Free CTAB solutions contained the same drug concentrations as the respective CTAB-MSN solutions. After 24 h, cell proliferation was assessed by adding MTT solution (50 μg/well), followed by incubation for 1 h. Suspensions from each culture were then dissolved in dimethyl sulfoxide (DMSO), and the optical density at 570 nm was determined using a microplate reader. This assay was repeated three times.

### 2.5. Statistical analysis

The results were analyzed using the Student’s *t*-test (two-tailed), with Prism GraphPad software. Differences with *P*-values < 0.05 were considered statistically significant. Data are expressed as mean ± SEM.

### 3. Results and discussion

CTAB-MSN were synthesized by a one-pot method in a basic aqueous solution. CTAB-MSN morphology and particle size were analyzed by transmission electron microscopy (TEM), which revealed nearly-monodispersed spheres of 110 to 170 nm in size (figure 1(a)). To verify the porous structure of CTAB-MSNs, a high-resolution TEM examination was performed. A uniform and orderly mesoporous structure was observed, with pore sizes estimated to be approximately 2.6 nm (figure 1(b)).

The fourier transform infrared (FTIR) spectra of CTAB, CTAB-MSNs, and MSNs are shown in figure 2. For CTAB-MSN, the characteristic peaks at 2922 cm⁻¹ and 2850 cm⁻¹ correspond to the C–H bond stretching vibration from CTAB, and the peak at 1087 cm⁻¹ represents the presence of the Si–O–Si bond in MSNs [9, 19]. The strong absorption bands at 2922 cm⁻¹ and 2850 cm⁻¹ for CTAB-MSN indicated that many CTAB molecules were embedded inside the CTAB-MSN nanopores.

To further quantify CTAB in the CTAB-MSNs, thermal gravimetric analysis (TGA) was performed. The TGA thermogram of CTAB-MSN in air (figure 3) revealed two weight loss stages. The first stage, between 30 and 200 °C, was due to the evaporation of physically-absorbed high boiling point solvent in the nanopores. The second weight loss, between 200 and 700 °C, corresponded to the decomposition and burning of CTAB, indicating approximately 37.2% of CTAB in the CTAB-MSNs.
To determine the cytotoxicity of MSNs on the growth of HepG2 cells, we performed MTT assays on these cells. Treatment of HepG2 cells with different concentrations of MSNs for 24 h revealed no significant effects on cell proliferation, even at higher MSN concentrations (figure 4). The results confirmed that nanomaterials possess slight cytotoxicity, indicating the superior biocompatibility of blank carriers.

To explore the potential of CTAB-MSNs for HepG2 cell therapy, stable concentrations of CTAB-MSNs were assessed and compared to free CTAB. CTAB-MSNs exhibited superior inhibition of cell proliferation compared to free CTAB.
to free drugs (figure 5). CTAB-MSNs also potently inhibited the proliferation of HepG2 cells, even at low concentrations. At the same drug concentration (2.5 μg ml⁻¹), the inhibition rate of CTAB-MSNs was approximately 50%, while free CTAB had no effect on cell growth. Free CTABs needed to be present at high concentrations to inhibit HepG2 cells. These results suggest that MSNs loaded with CTABs inhibit hepatocarcinoma cell growth better than free CTABs. CTAB-MSNs also induced morphological changes in the HepG2 cells (figure 6).

4. Conclusions

We successfully synthesized MSNs loaded with CTAB and demonstrated their efficacy as anticancer materials. The resultant CTAB-MSNs displayed uniform pore structure, high surface area, and high drug-loading capacity. The loading efficiency of CTAB on CTAB-MSNs was approximately 37.2% by weight. Cell viability assays revealed the superior anticancer activity of CTAB-MSNs compared to free CTAB. Our results suggest CTAB-MSNs have the potential to be advantageous over conventional therapies for HCC.

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Data availability

The data used to support the findings of this study are included within the article.

Conflict of interest

The authors declared that they have no conflicts of interests related to this work. We declare that we do not have any commercial or associative interest that represent a conflict of interest in connection with the work submitted.
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