Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Preparation of cupric sulfate-based self-emulsifiable nanocomposites and their application to the photothermal therapy of colon adenocarcinoma

Ja Seong Koo [a, 1], Song Yi Lee [a, 1], Suyeong Nam [a], Md Obyedul Kalam Azad [b], Minju Kim [c], Kwangyeol Kim [c], Byung-Jo Chae [c], Wie-Soo Kang [b], Hyun-Jong Cho [a, *]

[a] College of Pharmacy, Kangwon National University, Chuncheon, Gangwon, 24341, Republic of Korea
[b] Department of Bio-Health Technology, College of Biomedical Science, Kangwon National University, Chuncheon, Gangwon, 24341, Republic of Korea
[c] Department of Animal Resources Science, College of Animal Life Sciences, Kangwon National University, Chuncheon, Gangwon, 24341, Republic of Korea

ARTICLE INFO

Article history:
Received 28 June 2018
Accepted 1 July 2018
Available online xxx

Keywords:
Colon adenocarcinoma
Cupric sulfate
Hot-melt extrusion
Nanocomposite
Photothermal therapy

ABSTRACT

Nanocomposites (NCs) of cupric sulfate monohydrate ($CuSO_4$) were fabricated by hot-melt extrusion (HME) system equipped with twin screws. Micron-sized bulk powder of $CuSO_4$ was dispersed in the mixture of surfactants (Span 80 and Tween 80) and hydrophilic polymer (polyethylene glycol (PEG) 6000) by HME process. Reduction of surface tension by surfactants and homogeneous dispersion in hydrophilic polymer along with HME technique were introduced to prepare $CuSO_4$ NCs. Dispersion of $CuSO_4$ NCs exhibited approximately 204 nm hydrodynamic size, unimodal size distribution, and positive zeta potential values. Encapsulation of $CuSO_4$ in $CuSO_4$ NCs and the physicochemical interactions between $CuSO_4$ and pharmaceutical excipients were investigated by solid-state studies. Of note, $CuSO_4$ NCs group exhibited higher antiproliferation efficacies, compared with bulk $CuSO_4$, in Caco-2 (human adenocarcinoma) cells at 75 and 100 μg/mL $CuSO_4$ concentrations ($p < 0.05$). Also, near-infrared laser irradiation to $CuSO_4$ NCs group elevated the antiproliferation efficacies, compared with non-irradiation group, in Caco-2 cells. After intravenous injection in mice, $CuSO_4$ NCs did not show severe *in vivo* toxicities. Developed $CuSO_4$ NCs can be one of promising candidates of photothermal therapeutic agents for colon cancers.

1. Introduction

Copper (Cu) is one of essential trace dietary minerals for human body as well as other plants and animals [1,2]. After permeation across the gastrointestinal membrane, most portion was bound to metallothionein in the liver and released Cu will be incorporated into ceruloplasmin [3]. The deficiency of Cu may induce hematological disorders (*e.g.,* anemia and neutropenia), bone-related diseases (*e.g.,* osteoporosis), insufficient growth, higher rate of infection, and disorders in the glucose and cholesterol metabolism [4,5]. Thus, sufficient supplementation of Cu has been regarded as crucial for the maintenance of normal biological functions. The biological functions of Cu salt forms (*e.g.,* Cu_2S, CuS, Cu_2O, CuO, $CuSO_4$, and $CuCl_2$) and their carriers (*e.g.,* nanoparticles) have been elucidated [6–8].

The micron size of bulk powder of Cu may interrupt its biomedical application *via* an intravenous administration. As one of top-down methods for particle size reduction, hot melt extrusion (HME) technique was introduced to make colloidal particles of trace elements in our previous study [9]. Zinc sulfate ($ZnSO_4$) was dispersed in Soluplus by HME process and nano-sized particles of $ZnSO_4$ were prepared [9]. Generally, HME technique has been widely used for the preparation of solid dispersion formulation with poorly water-soluble drugs (*i.e.,* small organic compounds) [10,11]. Compounding and extruding processes of HME may contribute to the homogeneous dispersion of target molecules in the pharmaceutical excipients. In this study, surfactants and hydrophilic polymer were used for HME processing of $CuSO_4$ to prepare colloidal dispersion in the aqueous environment.

Among diverse salts and hybrid types of Cu, $CuSO_4$ has been used as an anticancer agent based on the higher mitochondrial

* Corresponding author.
E-mail address: hjcho@kangwon.ac.kr (H.-J. Cho).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bbrc.2018.07.002
0006-291X/

reactive oxygen species (ROS) generation and apoptotic event [12]. Cu is one of metals with high electrical and thermal conductivity. Thus, it can be used for photothermal therapy (PTT) of cancers after laser irradiation [13–15]. Near-infrared (NIR) ranged laser ablation to metal-based nanomaterials may generate heat energy [16]. The maintenance of malignant tumor tissue over 43 °C can be the principal mechanisms of PTT [16]. That heat can induce the irreversible damage of cancer cells and suppress their growth [16]. Nanocarriers of Cu salts and its hybrid forms (*e.g.,* $CuCl_2$, CuS, and CuSe) have shown anticancer efficacies against several cancer cells [15,17–22]. In this study, the micron size of bulk powder of $CuSO_4$ was reduced to nano-size range by HME process and the aids of surfactants and hydrophilic polymer. Then, the PTT efficacies of those $CuSO_4$ nanocomposites (NCs) against colon adenocarcinoma cells were demonstrated.

2. Materials and methods

2.1. Materials

$CuSO_4 \cdot H_2O$ ($CuSO_4$) was supplied by TMC Co., Ltd. (Anyang, Korea). Span 80 and Tween 80 were obtained from Daejung Chemical & Metals Co., Ltd. (Siheung, Korea). Polyethylene glycol (PEG) 6000 was acquired from Samchun Pure Chemical Co., Ltd. (Pyeongtaek, Korea). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin-streptomycin (Pen Strep) were obtained from Gibco Life Technologies, Inc. (Grand Island, NY, USA). All other reagents were of analytical grade.

2.2. Fabrication and characterization of $CuSO_4$ NCs

Prior to the feeding step of materials, $CuSO_4$, Span 80, Tween 80, and PEG 6000 were blended at 20:12:4:64 weight ratio. That solid mixture was conveyed to the feed hopper at a 45 g/min speed. Hot-melt extruder (STS-25HS, Hankook E.M. Ltd., Pyeongtaek, Korea) installed with the twin-screw system and a round-shaped die (1 mm diameter) was used for the extrusion of input materials [9]. The temperatures of barrel and die were 55 °C and 60 °C, respectively. The screw speed was set at 150 rpm in this process. Extrudates were cooled down and pulverized by the HBL-3500S grinder (Samyang Electronics Co., Gunpo, Korea).

The particle characteristics of $CuSO_4$ NCs dispersion in distilled water (DW) were tested. The mean diameter, polydispersity index, and zeta potential values were measured by dynamic light scattering (DLS) and laser Doppler methods (ELS-Z1000; Otsuka Electronics, Tokyo, Japan). At fixed $CuSO_4$ concentration, the hydrodynamic size of dispersion of inorganic salts ($CuSO_4$), physical mixture ($CuSO_4$:Span 80:Tween 80:PEG 6000 = 20:12:4:64, weight ratio), and HME formulation ($CuSO_4$ NCs) was measured. The morphology of $CuSO_4$ NCs (dispersed in DW) was observed by transmission electron microscopy (TEM). The aliquot of $CuSO_4$ NCs dispersion was put onto the copper grid with film and dried for 10 min. Dried samples were observed by TEM (JEM 1010; JEOL, Tokyo, Japan). The content of Cu in $CuSO_4$ NCs was quantitatively analyzed by inductively coupled plasma-optical emission spectrometry (ICP-OES; Optima 7300 DV, PerkinElmer, Inc., Waltham, MA, USA). Before ICP-OES analysis, $CuSO_4$ NCs were dissolved in nitric acid for their digestion.

2.3. Solid-state studies

2.3.1. Fourier-transform infrared (FT-IR) analysis

To investigate the chemical interactions between $CuSO_4$ and other pharmaceutical excipients, FT-IR spectra of $CuSO_4$ and $CuSO_4$ NCs were obtained with a Frontier™ FT-IR spectrometer (PerkinElmer Inc., Buckinghamshire, UK). Attenuated total reflectance (ATR) mode was used to acquire FT-IR data. Transmittance (%) of each group was scanned in 400–4000 cm^{-1} range.

2.3.2. X-ray photoelectron spectroscopy (XPS) method

The elemental composition and chemical state of $CuSO_4$ and $CuSO_4$ NCs were measured by XPS (K-Alpha™+, Thermo Fisher Scientific, East Grinstead, UK) in the Central Laboratory of Kangwon National University. Percentages of each atom in $CuSO_4$ and $CuSO_4$ NCs were quantitatively determined. The type of source gun was Al K_α X-ray and the spot size was 400 μm.

2.3.3. X-ray diffractometry (XRD) study

Crystal structure and physicochemical characteristics of $CuSO_4$ and $CuSO_4$ NCs were investigated by a Philips X'Pert PRO MPD diffractometer (PANalytical Corp., Almero, Netherlands). Intensity (counts) was measured in 10–80° of 2θ range. The step size and scan step time were designed as 0.013° and 8.67 s, respectively. Generator conditions were set as 30 mA and 40 kV.

2.3.4. Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) tests

Thermal properties of $CuSO_4$ and $CuSO_4$ NCs were tested by DSC/TGA analyzer (SDT Q600, TA instruments, New Castle, DE, USA). Weight (TGA), heat flow (DSC), and derivative weight (derivative thermogravimetry (DTG)) according to the temperature (50–800 °C) with nitrogen (N_2) were measured.

2.4. In vitro photothermal efficacy test

Thermal ablation effects of $CuSO_4$ and $CuSO_4$ NCs were tested and their irradiation time-dependent temperature profiles were recorded. $CuSO_4$ and $CuSO_4$ NCs were dispersed in DW at 50 μg/mL $CuSO_4$ concentrations and NIR laser with 808 nm wavelength and 1, 2, and 5 W/cm^2 laser power was irradiated to those dispersions for 15 min. The images and maximum temperatures were observed by thermal camera (Compact PRO, Seek Thermal, Inc., Santa Barbara, CA, USA).

2.5. In vitro anticancer activity test

Antiproliferative efficacies of $CuSO_4$ and $CuSO_4$ NCs were tested in Caco-2 cells by a colorimetric method. Caco-2 cells were purchased from the Korean Cell Line Bank (Seoul, Korea). Caco-2 cells were cultured in DMEM supplemented with FBS (10%, v/v) and penicillin-streptomycin (1%, v/v) in 95% relative humidity and 5% CO_2 atmosphere at 37 °C. Caco-2 cells were seeded in 96-well plate at a density of 5.0×10^3 cells and they were incubated at 37 °C for 24 h. $CuSO_4$ and $CuSO_4$ NCs were applied to the cells at 10, 25, 50, 75, 100, and 250 μg/mL $CuSO_4$ concentrations and they were incubated for 48 h. Cells with samples were submitted to the NIR laser irradiation (808 nm wavelength) at 2 W/cm^2 for 3 min. After removing each sample, MTS-based CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay Reagent (Promega Corp., Fitchburg, WI, USA) was added to the cells and they were incubated at 37 °C according to the manufacturer's guideline. The absorbance of each sample was read at 490 nm with a multi-mode microplate reader (SpectraMax i3, Molecular Devices, Sunnyvale, CA, USA) and the cell viability was calculated by comparison with that of control (no treatment) group.

2.6. In vivo toxicity tests

Toxicity of $CuSO_4$ NCs was evaluated in ICR mouse (male, 20 g of average body weight; Orient Bio, Sungnam, Korea) after

Please cite this article in press as: J.S. Koo, et al., Preparation of cupric sulfate-based self-emulsifiable nanocomposites and their application to the photothermal therapy of colon adenocarcinoma, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.07.002