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Preparation and characterization of water-soluble albumin-bound curcumin nanoparticles with improved antitumor activity

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ABSTRACT

Curcumin (CCM), a yellow natural polyphenol extracted from turmeric (*Curcuma longa*), has potent anti-cancer properties as has been demonstrated in various human cancer cells. However, the widespread clinical application of this efficient agent in cancer and other diseases has been limited by its poor aqueous solubility and bioavailability. In this study, we prepared novel CCM-loaded human serum albumin (HSA) nanoparticles (CCM-HSA-NPs) for intravenous administration using albumin bound technology. Field emission scanning electron microscopy (FE-SEM) and dynamic light scattering (DLS) investigation confirmed a narrow size distribution in the 130–150 nm range. Furthermore, CCM-HSA-NPs showed much greater water solubility (300-fold) than free CCM, and on storage, the biological activity of CCM-HSA-NPs was preserved with negligible activity loss. In vivo distributions and vascular endothelial cells transport studies demonstrated the superiority of CCM-HSA-NPs over CCM. Amounts of CCM in tumors after treatment with CCM-HSA-NPs were about 14 times higher at 1 h after injection than that achieved by CCM. Furthermore, vascular endothelial cell binding of CCM increased 5.5-fold, and transport of CCM across a vascular endothelial cell monolayer by Transwell testing was 7.7-fold greater for CCM-HSA-NPs than CCM. Finally, in vivo antitumor tests revealed that CCM-HSA-NPs (10 or 20 mg/kg) had a greater therapeutic effect (50% or 66% tumor growth inhibition vs. PBS-treated controls) than CCM (18% inhibition vs. controls) in tumor xenograft HCT116 models without inducing toxicity. We attribute this potent antitumor activity of CCM-HSA-NPs to enhanced water solubility, increased accumulation in tumors, and an ability to traverse vascular endothelial cell.

1. Introduction

Curcumin (CCM) is a low-molecular-weight natural polyphenol present in turmeric (*Curcuma longa*) with low intrinsic toxicity but a wide range of pharmacological activities, which include antitumor, antioxidant, anti-amyloid, and anti-inflammatory properties (Maheshwari et al., 2006). CCM is a potent inhibitor of NF-κB, a transcription factor implicated in the pathogeneses of several malignancies (Singh and Aggarwal, 1995), and also inhibits the productions of various cytokines, including tumor necrosis factor-α and interleukin-1β (Abe et al., 1999). Pre-clinical studies conducted on CCM have demonstrated that it inhibits carcinogenesis in a number of cell lines, including breast, cervical, colon, gastric, hepatic, leukemia, oral epithelial, ovarian, pancreatic, and prostate cancer cell lines (Aggarwal et al., 2003). Accordingly, there is interest in the clinical development of this compound as an anti-cancer agent (Sharma et al., 2005).

However, despite its promising anti-cancer properties, the extremely low water solubility of CCM limits its bioavailability and clinical efficacy, for example, its serum concentrations and tissue distributions are low, and furthermore, it is rapidly metabolized and thus has a short half-life (Anand et al., 2007). To address these problems, attempts have been made to encapsulate CCM in liposomes (Li et al., 2007; Kunwar et al., 2006), polymeric nanoparticles (Bisht et al., 2007), lipid-based nanoparticles (Sou et al., 2008), biodegradable microspheres (Kumar et al., 2002), cyclodextrin (Salmaso et al., 2007), and in hydrogel (Vemula et al., 2006).

Nanoparticles have been used as effective delivery tools to enhance the tumoricidal activities of anti-cancer drugs. Such delivery systems offer many benefits due to their tumor-targeting abilities, internalization efficiencies, and sometimes, escape from multi-drug resistance (Byrne et al., 2008; Song et al., 1997;

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Williams et al., 2003). Human serum albumin (HSA) is an excellent nanoparticle carrier because it is non-toxic and non-immunogenic. Furthermore, albumin bound nanoparticle technology does not require surfactants or polymeric materials to preparation. Accordingly, it is believed that HSA nanoparticles are likely to be well tolerated in vivo (Desai et al., 1999; Chen et al., 2008; Segura et al., 2007). Furthermore, albumin is thought to facilitate the endothelial transcytosis of unbound and albumin-bound plasma constituents to the extravascular space. This process is initiated by the binding of albumin to the endothelial cell surface to the 60-kDa glycoprotein (gp60) receptor (albondin), which in turn results in the binding intracellular protein (caveolin-1) by gp60 and invagination of the cell membrane to form transcytotic vesicles, referred to as caveolae (John et al., 2003; Minshall et al., 2002; Vogel et al., 2001). This efficacy conferred by the use of an albumin carrier is supported by the findings of several clinical studies, for example, on Albunex™ (Feinstein et al., 1990; Geny et al., 1993) and Abraxane™ (Damascelli et al., 2001; Ibrahim et al., 2002).

Here, we describe a CCM-loaded HSA nanoparticle (CCM-HSA-NP) using albumin bound nanoparticle technology. We also present the results of its physicochemical and pharmaceutical characterizations, its biologic and antitumor activities, biodistribution, and transport of vascular endothelial cells in vitro and in vivo.

2. Materials and methods

2.1. Materials and animals

Curcumin (CCM) (purity >99%), HSA (fraction V, purity 96–99%, 65 kDa) and all other chemicals were obtained from Sigma–Aldrich (St. Louis, MO), and were of reagent-grade, and were used as received. Cell lines were obtained from the Korean Cell Line Bank (Seoul, Korea) or from the ATCC (Manassas, VA). Animals were obtained from the Hanlim Experimental Animal Laboratory (Seoul, Korea), and were cared for according to the National Institute's of Health (NIH) guidelines for the care and use of laboratory animals (NIH publication 80-23, revised 1996).

2.2. Preparation of CCM-HSA-NPs

CCM-HSA-NPs were prepared using nanoparticle albumin bound technology (Desai et al., 1999). Briefly, 1000 mg of HSA was dissolved in 50 ml of water saturated with chloroform. Separately, 150 mg CCM was dissolved in 3 ml of chloroform saturated with water. These two solutions were then mixed and homogenized (EmulsiFlex-C5 homogenizer, Canada) at 20,000 psi for nine cycles. The resulting colloid was rotary evaporated to remove chloroform at 25 °C for 15 min under reduced pressure. The nanoparticles obtained were then filtered through a 0.25 μm membrane syringe filter and solvent was removed by lyophilization for 48 h at −70 °C. The CCM-HSA-NP powder obtained was vacuum-dried for 24 h and stored at 4 °C until required.

2.3. HPLC analysis of CCM

CCM levels were determined by high-performance liquid chromatography (HPLC) using a Waters Nova-Pak C18 column (150 mm × 3.9 mm, 5 μm particle size) at ambient temperature. The mobile phase consisted of 40% THF and 60% water containing 1% citric acid, adjusted to pH 3 with concentrated KOH solution (v/v), as previously described (Cooper et al., 1994). The system was run isocratically at a flow rate of 1 ml/min and CCM was detected at 420 nm.

2.4. Characterization of CCM-HSA-NPs

To determine CCM loading efficiencies in NPs, 1 mg of CCM-HSA-NPs was dissolved in 10 ml of ethyl acetate/propanol (9:1, v/v) and sonicated for 30 min to extract CCM completely. CCM concentration in solution was measured by HPLC, and CCM loading efficiency was defined as CCM content (%, w/w) = (CCM weight in nanoparticles/total nanoparticle weight) × 100. The mean particle diameters and size distributions were determined by dynamic light scattering (DLS; Brookhaven Instrument Co., NY, USA) at 633 nm using a detection angle of 90°. CCM-HSA-NP zeta potentials were determined by photon correlation spectroscopy (Malvern Zetasizer, Malvern Instruments, UK). Measurements were performed at 25 °C, and the data reported are the mean values of triplicate determinations. The shapes of CCM-HSA-NPs were determined by field emission scanning electron microscopy (FE-SEM), using a LEOSUPRA 55 GENESIS 2000 unit at an accelerating voltage of 15 kV.

2.5. Water solubility study

To determine the water solubility of CCM-HSA-NPs, 10 mg of CCM or an equivalent amount of CCM-HSA-NPs was added to 1 ml of deionized water. Mixtures were vortexed for 5 min, sonicated for 1 min, and centrifuged at 20,000 rpm for 5 min. Supernatants were mixed with ethyl acetate (1:1, v/v) to extract CCM. Finally, CCM levels were determined by HPLC at 420 nm.

2.6. In vitro cytotoxicity

The cytotoxicity of CCM-HSA-NPs was investigated in human colon tumor cells and in human pancreatic carcinoma cells (HCT116 or MiaPaCa2, ATCC, Manassas, VA). The cell lines were characterized according to the guidelines issued by the ATCC (ATCC Technical Bulletin No. 8, 2008). Cells were maintained in Dulbecco's modified Eagle's medium, supplemented with 10% (v/v) FBS (fetal bovine serum) containing 1% penicillin/streptomycin. For dose-dependent cytotoxicity assays, cells were seeded in 96-well plates at 1×10^4 cells/well and pre-incubated for 24 h. Media were replaced with fresh serum-free DMEM and then pre-determined amounts of HSA-NPs, CCM, or CCM-HSA-NPs were added for 72 h. The in vitro cytotoxicities of HSA-NPs, CCM, and CCM-HSA-NPs were determined using MTT assays.

2.7. Storage stability study

Storage stability was determined according to the guidelines issued by the International Conference on Harmonization (ICH) (1993). Briefly, sealed vials of freshly prepared freeze-dried CCM-HSA-NPs were placed in a stability chamber maintained at 25 °C and 60% RH for up to 3 months. CCM-HSA-NPs were analyzed for size and polydispersity index (PDI) at 1-month intervals.

2.8. In vivo biodistribution of CCM-HSA-NPs

Biodistributions were determined as previously described (Pan et al., 1999). Briefly, C57BL6 mice bearing B16F10 murine melanomas cells (Korean Cell Line Bank (KCLB), Seoul, Korea) (100 mm^3) were prepared, and tissues and blood samples were obtained at pre-determined times after the i.v. administration of CCM or CCM-HSA-NPs (20 mg/kg). CCM was solubilized using cremophor EL/ethanol. The tissues were removed, minced with a scissors, and homogenized in 3 volumes of phosphate buffer (pH 3) using a polytron. Plasma was collected from heparinized blood samples by centrifugation at 4300 × *g* for 10 min. An aliquot of homogenate or plasma then was transferred to a glass stoppered centrifuge tube, acidified to pH 3 with 6 N HCl, and extracted with

Table 1
Preparation of CCM-HSA-NPs.[a]

Name of NPs	HSA concentration (%, w/w)	CCM concentration (mg/ml)	Organic phase:water (v/v)	Particle size (nm)	Zeta potential (mV)	CCM content (%)
1	2	25	1:19	119.4 ± 1.7	−22.2 ± 0.3	4.3 ± 1.2
2	2	50	1:19	135.5 ± 2.9	−23.4 ± 1.2	7.2 ± 2.5
3	2	75	1:19	143.3 ± 2.4	−24.6 ± 1.7	11.8 ± 1.1
4	2	50	1:9	149.2 ± 1.2	−23.6 ± 0.4	14.1 ± 1.7
5	2	50	1:29	136.7 ± 3.4	−23.9 ± 0.7	4.5 ± 0.6
6	2	50	1:49	124.5 ± 2.2	−25.1 ± 0.2	3.9 ± 0.3
7	2	50	1:74	121.6 ± 3.2	−25.3 ± 0.9	3.1 ± 0.4

[a] Data are means ± SDs ($n = 3$).

equal volumes of ethyl acetate/propanol (9:1, v/v) by mechanical shaking for 6 min. Samples were then centrifuged at 5000 × *g* for 20 min, and top layers were subjected to HPLC. The extraction efficiency of this method for CCM was 95%. Levels of CCM were measured in lung, liver, kidney, spleen, heart, and tumor homogenates in the same manner.

2.9. Binding and transport studies using vascular endothelial cells

Binding and transport studies were performed as previously described (Desai et al., 2006). To quantify CCM-HSA-NP binding to vascular endothelial cells, human umbilical vascular endothelial cells (HUVEC, ATCC, Manassas, VA) were grown on 6-well plates. CCM or CCM-HSA-NPs were then added at final concentrations of 25, 50, 100, 200, or 400 μg of CCM in PEM buffer (50 mM PIPES, 2 mM EGTA, 2 mM $MgCl_2$) for 1 h at 37 °C, washed with PEM buffer 2–3 times, and lysed with cell lysis buffer (0.5 N NaOH with 1% SDS). CCM was extracted from lysates with ethyl acetate/propanol (9:1, v/v). Extracts were separated by centrifugation at 5000 × *g* for 10 min and CCM was analyzed by HPLC at 420 nm.

To investigate the ability of CCM-HSA-NPs to cross a vascular endothelial cell monolayer, a human lung microvascular endothelial cell line (HLMEC, ATCC CCL211, ATCC Rockville, MD) in EBM-PRF medium (Cambrex, East Rutherford, NJ) was seeded at 1×10^6 per insert to the top membrane surface in a Transwell unit (Falcon HTS FluoroBlok Inserts, BD Biosciences, San Jose, CA). Twenty-four hours later, the cells were incubated with EBM-PRF medium supplemented with 5% HSA and either 0 or 10 mM β-methyl cyclodextrin (BMC) at 37 °C. Transport was initiated by adding CCM or CCM-HSA-NPs at a final CCM concentration of 200 μg in medium supplemented with 5% HSA to the upper chamber. The transport of CCM across the endothelial barrier into the bottom chamber was analyzed 2, 4, 6, 8 and 10 h later by HPLC as described above.

2.10. In vivo antitumor activity

Balb/c nu/nu male mice (6–7 weeks old) were used as a tumor xenograft model. Tumors were established by inoculating HCT 116 or MiaPaCa2 cells (4×10^6 cells/mice, 50 μl injection) s.c. into a dorsal flank of each mouse. Five days after tumor cell inoculation, mice were administered CCM (10 mg/kg, i.v., solubilized in cremophor EL/ethanol) or CCM-HSA-NPs (10 or 20 mg/kg, i.v.) solubilized in saline every other day for 10 days (5 applications). Tumor diameters were measured using a digital caliper and tumor volumes (in mm^3) were calculated using the formula: tumor volume = length × $width^2$ × 0.5. To monitor for potential toxicities, body weights were measured every 2 days. The experiment was terminated at 18 days after injection.

2.11. Statistical analysis

Data are expressed as means ± SDs, and the Student's *t*-test was used throughout.

3. Results and discussion

3.1. Preparation and characterization of CCM-HSA-NPs

CCM-HSA-NPs were prepared using nanoparticle albumin bound technology (Desai et al., 1999). Albumin nanoparticles can be prepared by desolvation or emulsification followed by denaturation by heating or chemical crosslinking (Wartlick et al., 2004a,b; Dreis et al., 2007). However, these methods are unsuitable for water-insoluble drugs, and the final state of the albumin differs from that produced by nanoparticle albumin bound technology. During chemical crosslinking processes, amines or hydroxyls present in HSA are cross-linked nonspecifically, and during the heat denaturation process the structure of HSA is also irreversibly

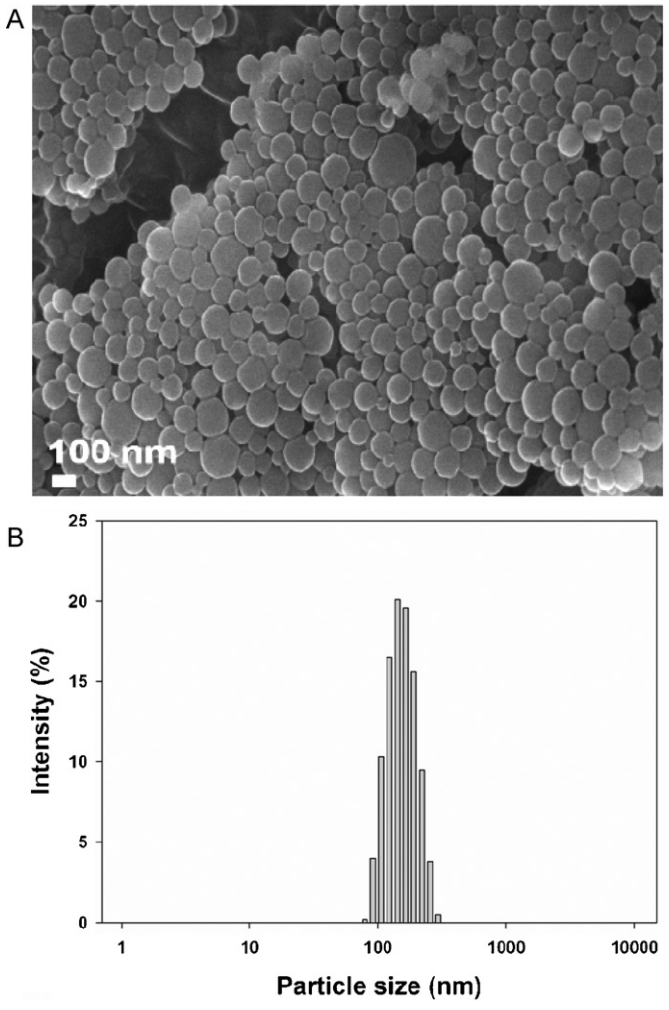

Fig. 1. Characterization of CCM-HSA-NPs. (A) FE-SEM photograph of CCM-HSA-NPs (Original magnification 30,000×). (B) Dynamic light scattering (DLS) result.

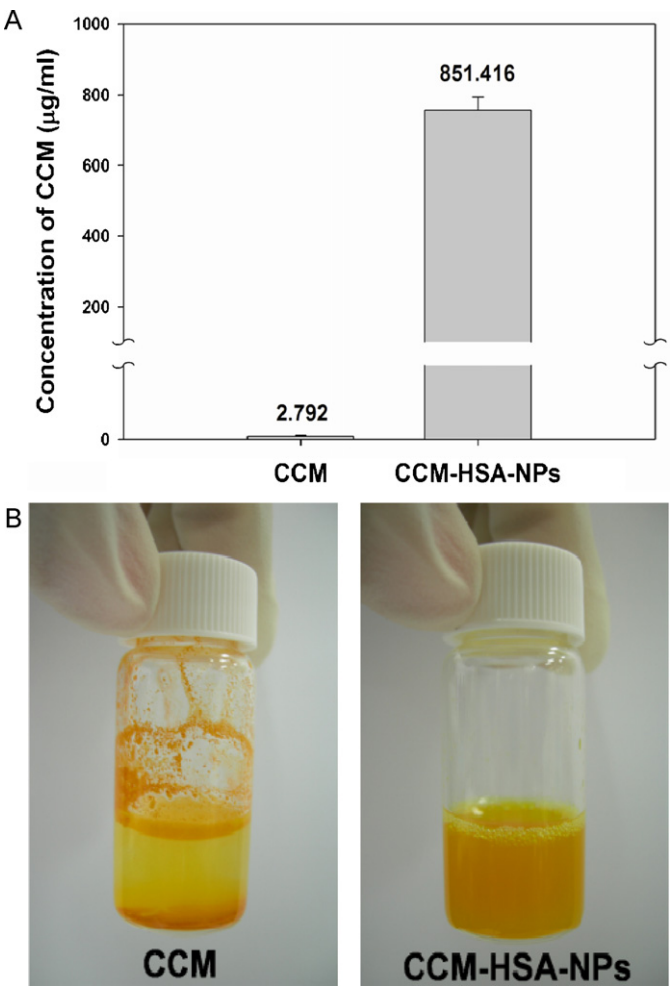

Fig. 2. Water solubilities of CCM and CCM-HSA-NPs. (A) HPLC determined solubilities of CCM and CCM-HSA-NPs in water ($n = 3$). Means ± SDs. (B) Photographs showing the insolubility of CCM and the solubility of CCM-HSA-NPs in water (10 mg/ml).

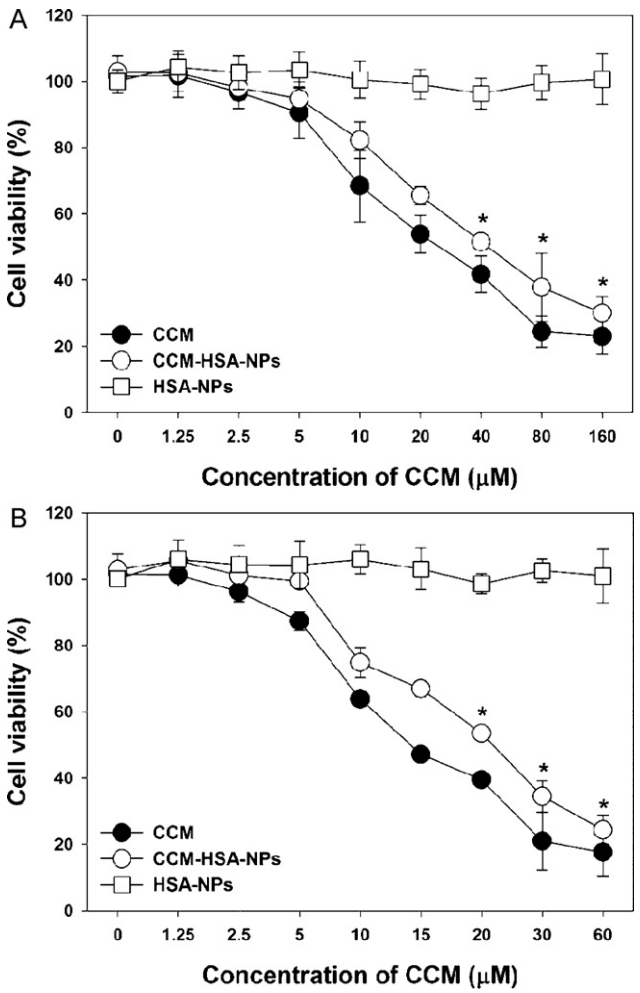

Fig. 3. In vitro cytotoxicities of CCM and CCM-HSA-NPs. (A) The cytotoxic effects of CCM and CCM-HSA-NPs on human colon tumor cells (HCT116) and on (B) human pancreatic carcinoma cells (MiaPaCa2) ($n = 6$). Means ± SDs, $^*p < 0.05$ compared to the CCM.

altered. In contrast, during the preparation of CCM-HSA-NPs, the sulfhydryl residues of HSA may be oxidized (and/or existing disulfide bonds may be disrupted) to form new, crosslinking disulfide bonds due to the localized heating associated with cavitation produced by the high-pressure homogenizer. However, the disulfide formation induced by homogenization does not substantially denature the HSA (Desai et al., 1999). Furthermore, no surfactants, polymers, or toxic solvents are used to produce CCM-HSA-NPs, and therefore, CCM-HSA-NPs were designed to be safe and suitable for intravenous usage.

The effects of CCM concentration and of the ratio of organic solvent to water (v/v) are shown in Table 1. When the concentration of CCM was increased, mean particle size and CCM loading in CCM-HSA-NPs also increased. However, when the ratio of organic solvent to water was decreased, both particle size and CCM loading decreased. To obtain CCM-HSA-NPs with a mean particle size smaller than 150 nm and with a satisfactory CCM loading, a HSA concentration of 2% (w/w), a CCM concentration of 50 mg/ml, and an organic phase/water to water ratio of 1:19 was used in this research. For three batches of CCM-HSA-NPs, the mean particles size was 135.5 ± 2.9 nm, the zeta potential was −23.4 ± 1.2 mV, and the CCM loading was 7.2 ± 2.5%. The resulting CCM-HSA-NPs had a mean size of 139.6 ± 2.5 nm as determined by dynamic light scattering (DLS). FE-SEM imaging showed that the particles were spherical and 130–150 nm in size (Fig. 1).

3.2. Water-solubility and cytotoxicity

The water solubility of CCM-HSA-NPs was determined by HPLC at 420 nm (Fig. 2A). The solubility of CCM in water was 2.792 μg/ml, whereas that of CCM-HSA-NPs was 851.416 μg/ml (quoted as CCM equivalents), which represented a 300-fold increase. This improved water solubility enabled use to replace the original cremophor EL/ethanol based formulation with saline for the in vitro and in vivo applications.

The biological activity of CCM-HSA-NPs was investigated using human colon tumor cells (HCT116) and human pancreatic carcinoma cells (MiaPaCa2) and compared with that of CCM. CCM-HSA-NPs showed only a slight reduction in cytotoxicity vs. CCM (IC_{50} values against HCT116 and MiaPaCa2 were 30.35 and 14.69 μM for CCM and 39.17 and 21.35 μM for CCM-HSA-NPs, respectively) (Fig. 3). Furthermore, HSA-NPs were not found to have any adverse effect on cell viability. These similar cytotoxicities of CCM-HSA-NPs and CCM (on a CCM equivalent basis) observed in the present study suggest that the molecular structure of CCM in CCM-HSA-NPs is substantially unchanged.

3.3. Storage stability

Various reports have been issued on the instabilities of nanoparticle formulation during storage. To evaluate the long-term

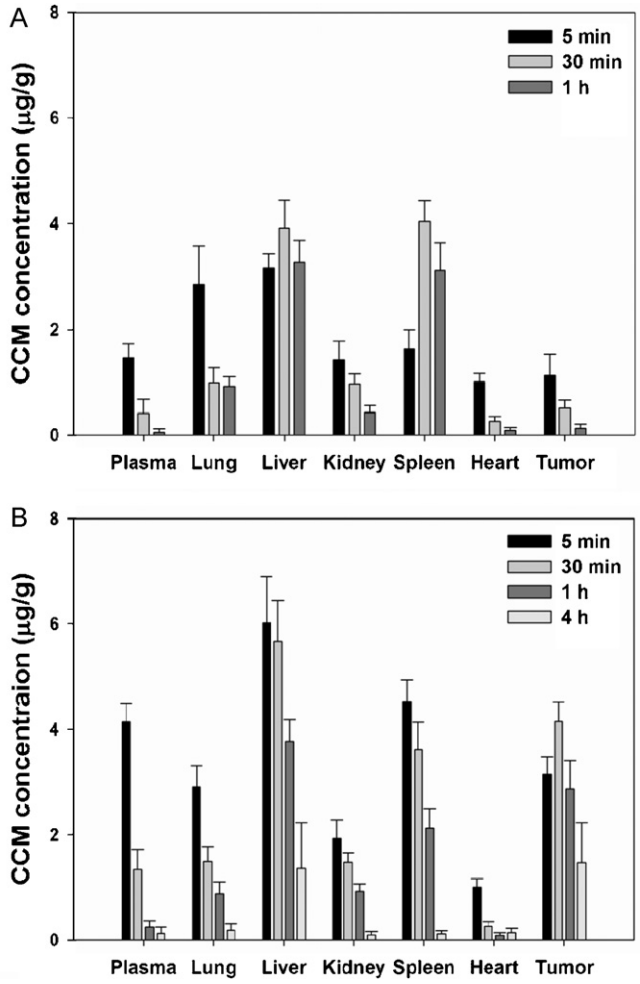

Fig. 4. In vivo biodistributions of CCM and CCM-HSA-NPs. (A) Tissue (plasma, lung, liver, kidney, spleen, heart, and tumor) distributions of CCM, and (B) of CCM-HSA-NPs in B16F10 murine melanomas cells bearing C57BL6 mice after treatment (20 mg/kg (CCM equivalents), i.v.) ($n = 5$). Means ± SDs.

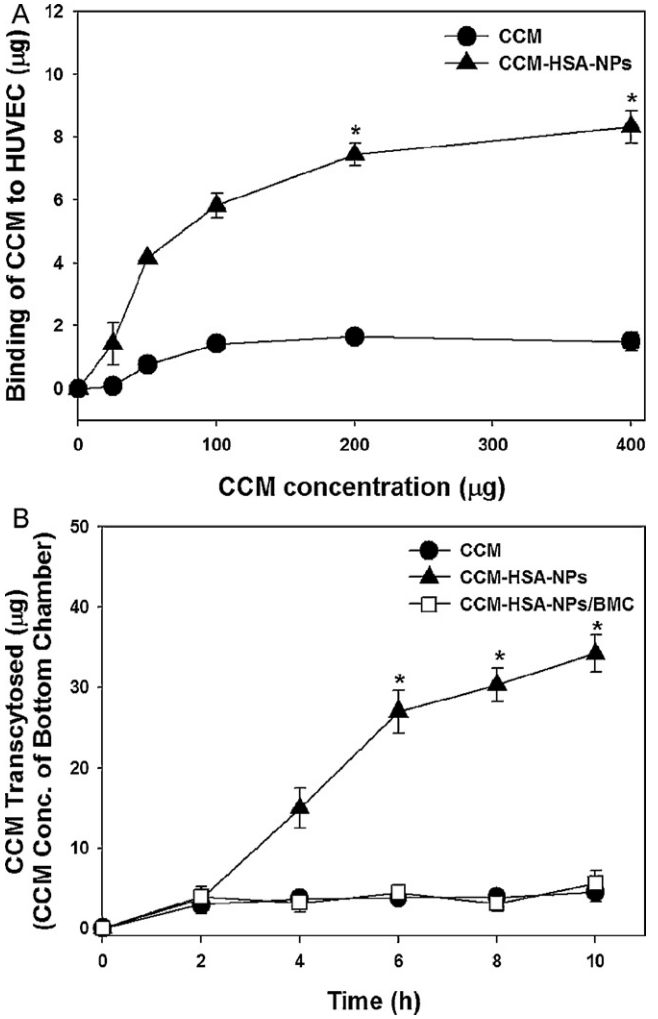

Fig. 5. Binding of CCM and CCM-HSA-NPs to and their transport through vascular endothelial cells. (A) Binding of CCM and CCM-HSA-NPs to human umbilical vascular endothelial cells (HUVECs) ($n = 3$). (B) Transcytosis of CCM and CCM-HSA-NPs ($n = 3$). Means ± SDs, $^*p < 0.05$ vs. CCM.

stability of our formulation studies were conducted according to the ICH guidelines (http://www.ich.org/cache/compo/363-272-1.html#Q1A(R2)). After 3 months of storage stability at 25 °C and 60% RH, freeze-dried CCM-HSA-NPs with an excipient appeared be stable as dried cakes showed no collapse or shrinkage. Furthermore, particle size and polydispersity index measurements indicated no change had occurred during storage stability testing (Table 2). Long-term stability is a major constraint that must be adequately satisfied, and freeze-drying is considered as a viable test of long-term stability. Thus, CCM-HSA-NPs were freeze-dried with no excipient, because HSA acts as a cryoprotectant and aids reconstitution.

3.4. In vivo biodistribution of CCM-HSA-NPs

To investigate the biodistribution of CCM-HSA-NPs in vivo, we administered CCM (formulated in cremophor EL/ethanol) and CCM-HSA-NPs (formulated in normal saline) to C57BL6 mice bearing B16F10 murine melanomas cells at an equivalent CCM dose of 20 mg/kg. It was found that CCM concentrations in tumor tissues were much higher in mice administered CCM-HSA-NPs, irrespective of time (Fig. 4). At 0.5 h after treatment, CCM was found mainly in normal tissues, that is, liver, spleen, lungs, and kidneys (in decreasing order). However, CCM-HSA-NP uptake was unremarkable in normal tissues other than liver, which is not surprising, as the liver generally shows high uptake than other organs, because most macromolecules larger than the renal cut-off size (10 nm) (Davis et al., 2008) are eliminated predominately via reticuloendothelial system uptake in the liver. Tumor accumulation of CCM was poor, but CCM-HSA-NP accumulation was remarkably higher. At 1 h after administration, CCM was gradually eliminated from all tissues and tumors. However, CCM tumor accumulation after CCM-HSA-NP administration was 14 times higher at 1 h after administration. Furthermore, at 4 h after administration, CCM tumor accumulation after CCM-HSA-NP administration still amounted to 1.44 μg/g. These findings show that CCM-HSA-NPs preferentially accumulate in tumors, probably because of the enhanced permeability and retention (EPR) effects and the ability of albumin to promote transport across endothelial cell.

Table 2
Storage stability of CCM-HSA-NPs ($n = 3$).[a]

Time point	Particle size (nm)	PDI
0	137.3 ± 1.5	0.31 ± 0.06
1 month	138.6 ± 2.4	0.32 ± 0.03
2 month	139.1 ± 1.9	0.32 ± 0.05
3 month	141.8 ± 1.3	0.33 ± 0.09

[a] Data are means ± SDs ($n = 3$), PDI: polydispersity index.

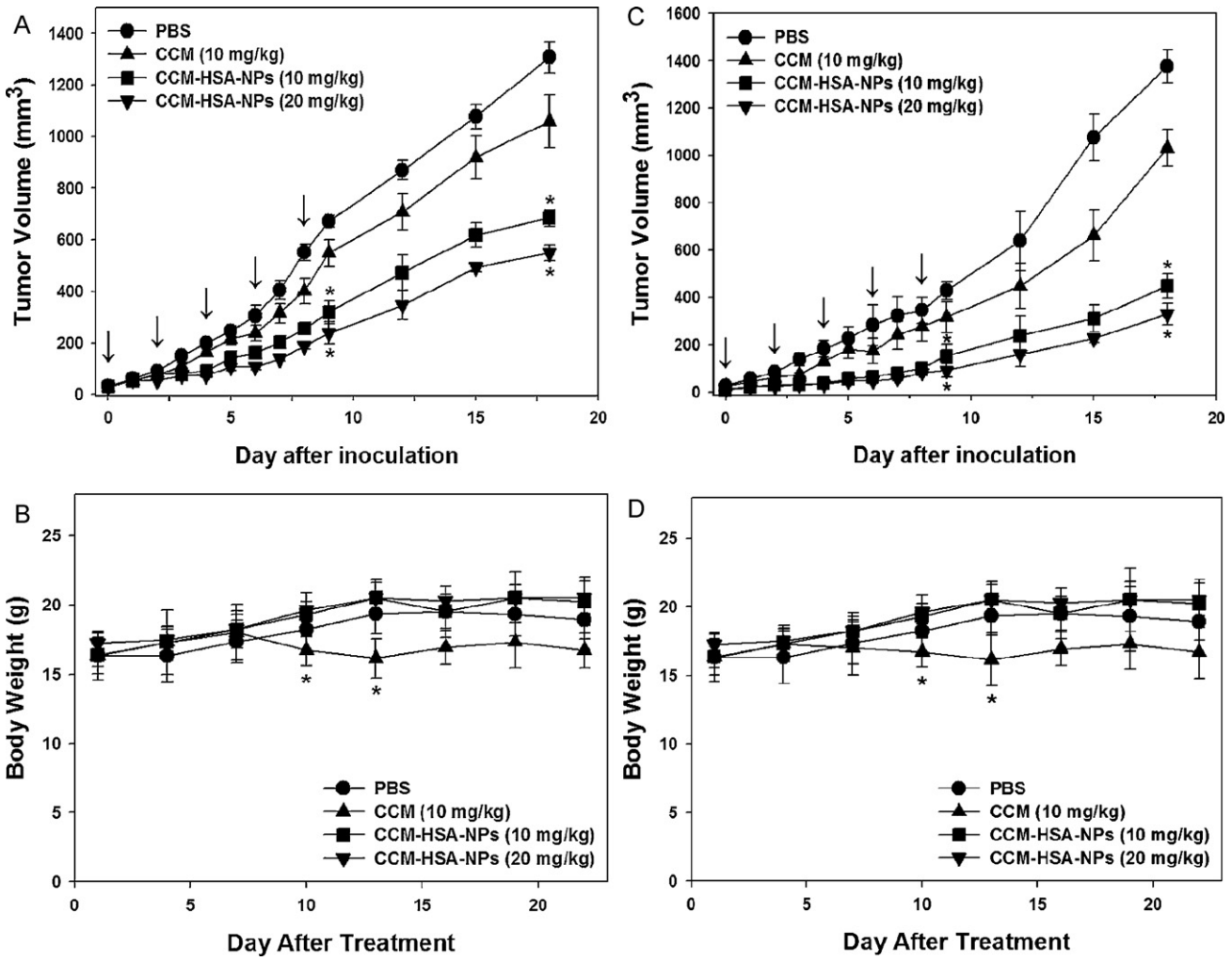

Fig. 6. In vivo antitumor activities and body weight changes of CCM (10 mg/kg) and CCM-HSA-NPs (10 or 20 mg/kg). (A) Antitumor activities and (B) body weight changes in human colon cancer (HCT116) xenograft models after the i.v. administration of CCM or CCM-HSA-NPs ($n = 5$). (C) Antitumor activities and (D) body weight changes in human pancreatic carcinoma (MiaPaCa2) xenograft models after i.v. administration of CCM or CCM-HSA-NPs ($n = 5$). Means ± SDs, $^*p < 0.05$ vs. PBS-treated controls.

3.5. Binding and transport studies in vascular endothelial cells

To investigate the potential mechanisms responsible for increased the accumulation of CCM in tumors from CCM-HSA-NPs, we examined their endothelial binding and transport characteristics. Endothelial binding of CCM increased 5.5-fold (Fig. 5A) and its transport across an endothelial cell monolayer increased 7.7-fold (Fig. 5B) for CCM-HSA-NPs as compared with CCM. Furthermore, the endothelial transcytosis of CCM-HSA-NPs was completely suppressed by β-methyl cyclodextrin (BMC) (Fig. 5B), an inhibitor of caveolar mediated transcytosis (Tiruppathi et al., 2004).

The transendothelial cell transport of albumin is mediated by gp60 (albondin) receptor and by caveolar transport (John et al., 2003; Schubert et al., 2001). Albumin binding to gp60 activates caveolin-1 and results in the formation of caveoli, which transport albumin and other plasma constituents across endothelial cells. Therefore, to identify the mechanisms responsible for the increased tumor accumulation of CCM by CCM-HSA-NPs, we compared the endothelial bindings and the abilities of CCM-HSA-NPs and CCM to transit endothelial cells. The increased abilities of CCM-HSA-NPs to bind and transit endothelial cells were striking, and both were inhibited by cotreatment with β-methyl cyclodextrin (BMC), a known inhibitor of caveolar transport (Kranenburg et al., 2001). Conventional thinking regarding drug transport to tumors has focused on passive transport via the fenestrations in tumor microvessels (Kranenburg et al., 2001). Our results suggest that the endothelial cells of tumor microvessels play an active role in transport of CCM-HSA-NPs from the vasculature to tumor interstitium via an albumin-based receptor-mediated pathway.

3.6. In vivo antitumor activity

To evaluate the antitumor activity of CCM-HSA-NPs in human colon cancer (HCT116) or human pancreatic carcinoma (MiaPaCa2) xenograft models in vivo, we examined tumor growths and body weight changes in nude mice treated with saline, CCM (10 mg/kg), or CCM-HSA-NPs (10 or 20 mg/kg). Five days after tumor cell injection, tumors had formed at all sites. The tumor growth rate was highest in saline-treated mice, and CCM was found to have a slight anti-tumor effect (18% and 26% tumor growth inhibitions for HCT116 and MiaPaCa2, respectively, on day 10 post-injection (at the end of the treatment), $p < 0.05$; 18%, 25% inhibition of tumor growth at day 18 (at the end of the study, $p < 0.05$)). However, CCM-HSA-NPs (10 or 20 mg/kg) dramatically suppressed the tumor growths of both HCT 116 and MiaPaCa2 derived tumors (52%, 64% or 64%, 78% (10 or 20 mg/kg) inhibition of tumor growth at day 10, respectively, $p < 0.05$; 47%, 58% or 67%, 76% inhibition of tumor growth at day 18, respectively, $p < 0.05$) (Fig. 6A and C). Furthermore, these suppressions were significantly greater than those observed for CCM. In addition, these

effects of CCM-HSA-NPs did not induce any detectable systemic cytotoxicity.

Toxicities were also assessed by monitoring the effects of treatments on body weights. Animals treated with CCM showed a decrease in body weight vs. PBS-treated controls, whereas animals administered CCM-HSA-NPs showed no appreciable reduction in body weight (Fig. 6B and D). These findings suggest that the i.v. administration of CCM-HSA-NPs is likely to have advantages over the current clinical CCM formulation, because it does not require the use of solubilizers like cremophor EL and ethanol.

4. Conclusions

In this study, CCM loaded nanoparticles (CCM-HSA-NPs) were prepared using nanoparticle albumin bound technology. CCM-HSA-NPs showed markedly greater water solubility (300-fold) than CCM, and this allowed us to eliminate cremophor EL and ethanol from the formulation, and thus, to avoid their toxic effects. The biological activity of CCM in CCM-HSA-NPs was found to be preserved in vitro. Furthermore, our in vivo tumor distribution and vascular epithelial cell transport studies demonstrate the superiority of CCM-HSA-NPs over CCM. Moreover, CCM-HSA-NPs showed better in vivo antitumor activity than CCM in a tumor xenograft animal model, with no observable toxicity. We attribute this potent antitumor activity of CCM-HSA-NPs to its enhanced water solubility and the ability to transit epithelial cells and localize in tumors. In addition, our findings show that HSA-based nanoparticle technology offers a promising drug delivery system for CCM in the treatment of cancer.

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