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# Influence of curcumin-loaded cationic liposome on anticancer activity for cervical cancer therapy

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ABSTRACT

The delivery of curcumin has been explored in the form of liposomal nanoparticles to treat various cancer cells. Since curcumin is water insoluble and an effective delivery route is through encapsulation in liposomes, which were modified with three components of DDAB, cholesterol and non-ionic surfactant. The purpose of this study was to establish a critical role of DDAB in liposomes containing curcumin at cellular response against two types of cell lines (HeLa and SiHa). Here, we demonstrate that DDAB is a potent inducer of cell uptake and cell death in both cell lines. The enhanced cell uptake was found on DDAB-containing liposome, but not on DDAB-free liposome. However, the cytotoxicity of DDAB-containing liposomes was high and needs to be optimized. The cytotoxicity of liposomal curcumin was more pronounced than free curcumin in both cells, suggesting the benefits of using nanocarrier. In addition, the anticancer efficiency and apoptosis effect of the liposomal curcumin formulations with DDAB was higher than those of DDAB-free liposomes. Therefore curcumin loaded liposomes indicate significant potential as delivery vehicles for the treatment of cervical cancers.



## 1. Introduction

Curcumin, the natural products obtained from the rhizome of turmeric (*Curcuma longa*), has been widely used as traditional medicine [1]. This is due to numerous medicinal properties of curcumin, which possesses antioxidation [2] and pleiotropic effects on the cancer cells through the modulation of multicellular signaling pathways including cell cycle, apoptosis, proliferation, invasion, angiogenesis, metastasis and inflammation [3]. Thus, curcumin has been considered as a traditional medicine exhibited therapeutic effect against cancers. Molecular targets and mechanisms of curcumin involved in treatment of cancers have documented [4–6]. Although curcumin influences numerous biochemical and molecular cascades and eventually inhibit growth of cancer cells, the use of curcumin is currently limited in clinical utility [7]. The main reason is its poor solubility in aqueous solvent. Furthermore, curcumin appears to be unstable at neutral and basic pH so that the soluble-curcumin molecules are sensitive at physiological pH. With these limitations, usage of curcumin in many preclinical and clinical studies often faced with low bioavailability of curcumin [8,9]. To enhance bioavailability and protect curcumin from degradation, nanoparticle-based curcumin drug formulation is considered as the promising approaches for cancer therapy [10]. Various types of nanocarriers have been developed including lipid-based and polymeric-based nanoparticles to increase the solubility and bioavailability of curcumin delivery [11–13]. The nanocarriers can enhance the uptake of curcumin into cancer cell [14]. In this study, we selected cationic liposome as a vehicle for curcumin intravenous delivery due to their biocompatibility, low cytotoxicity and low immunogenicity. Although the cationic liposome has been focused as a carrier for gene therapy [15], the use of cationic liposome for selective drug targeting on tumor cell has been purposed as rational and promising therapeutic approach as well [16]. The cationic liposomes have been shown to preferentially target the angiogenic endothelium of tumors [17]. Delivery of drug-loaded cationic liposome targets and destroys the vascular function which eventually limits the growth of the primary tumor and metastasis. Several preclinical studied have been evaluated the use of cationic liposome for delivery of chemotherapeutic drug delivery, especially paclitaxel [18,19]. However, the study of cationic liposome containing curcumin has not been widely investigated [20]. In this study we aim to enhance biological effect of curcumin by cationic liposomes, which were formulated through surface modification of liposome by various composition of didecyldimethylammonium bromide (DDAB), cholesterol and non-ionic surfactant (Montanov82®). The presence of non-ionic surfactant, with the Critical Micelle Concentration (CMC) value of 23 mM, is expected to reduce the agglomeration between liposome

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*via* steric repulsion of glucoside units as previously reported [21]. Cytotoxicity and apoptosis effect of DDAB-containing liposome against cervical cancer cells were also evaluated compared to DDAB-free liposome. Cellular internalization and cell interaction of liposome with human cervical cancer cells were determined.

## 2. Materials and methods

### 2.1. Materials

Soybean lecithin was from Degussa (Hamburg, Germany). Cocoyl glucoside surfactant (Montanov82®) was from Adinov (Bangkok, Thailand). Cholesterol was from Avanti Polar Lipids (Birmingham, USA). Didecyldimethylammonium bromide (DDAB) was from Sigma–Aldrich Chemical Company (St. Louis, USA). Phosphate buffer saline pH 7.4 (PBS; containing 137 mM NaCl, 2.7 mM KCl, 10 mM $Na_2HPO_4$; 2 mM $KH_2PO_4$), and Triton X-100 were from Merck (Merck, Germany). Curcumin was from Sigma–Aldrich (Buchs, Switzerland), Dulbecco's Modified Eagle's Medium (DMEM) and Minimum Essential Medium-Alpha (MEM-α) were from GIBCO Invitrogen (New York, USA). Fetal bovine serum (FBS) was from Biochrom AG (Berlin, Germany). Trypsin–EDTA, L-glutamine, penicilin G sodium, streptomicin sulfate, and amphotericin B were obtained from Invitrogen Corp. (New York, USA). MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide] was purchased from GIBCO Invitrogen (NY, USA). Dimethylsulfoxide (DMSO) was from Sigma–Aldrich, Inc. (Dorset, UK). HeLa (HPV type 18 positive cells) and SiHa (HPV type 16 positive cells) were from American Type Culture Collection (ATCC). Annexin V-FITC Apoptosis Detection Kit was obtained from NeXins Research BV (Rotterdam, the Netherlands). Distilled water was from ELGA (PureLab Ultra, Illinois, USA).

### 2.2. Cervical cancer cell cultivations

HeLa (HPV type 18 positive cells) and SiHa (HPV type 16 positive cells; ATCC HTB-35) were cultured in DMEM and MEM-α medium, respectively. The media were supplemented with 10% FBS containing 0.1 mM non-essential amino acids (100 μg/ml L-glutamine, 100 μg/ml streptomycin and 100 U/ml penicillin) at 37 °C in a humidified atmosphere containing 5% $CO_2$. Cell cultures were performed in 75 ml T-flasks. The media were changed every other day.

### 2.3. Formulation of free liposomes and curcumin-loaded liposomes

Liposomes were prepared by the conventional thin film hydration method [22]. In brief, a series of curcumin-loaded liposomes (Cur-liposomes) were prepared using various proportions of soybean lecithin, non-ionic surfactant (Montanov82®), cholesterol and DDAB in 10 ml mixture of chloroform-diethyl ether (2:1, v/v) as indicated in Table 1. The solvent was removed by rotary evaporation at 25 °C under 50–100 kg/cm$^2$ nitrogen flow to obtain a thin lipid film (approximately 15 h). The dried lipid film was rehydrated with PBS pH 7.4 and resuspended by shaking at 25 °C. The size of hydrated solution was decreased by discontinuous extruder Liposo-Fast™-10 (Avestin Inc, Ottawa, Canada) at 400 bar, 15 cycles with polypropylene membrene with 100 nm pore size (Millipore GmbH, Eschborn, Germany). The Cur-liposome was purified by ultracentrifuge (Beckman, Germany) at 80,000 rpm for 3 h for three times to remove free-curcumin and impurities [23]. The pellet of Cur-liposome was resuspened into PBS pH 7.4 buffer. All liposome formulations were freshly prepared for all assays.

### 2.4. Physicochemical characterization

Hydrodynamic diameter, polydispersity index (PDI) and zeta potential of liposomes was determined by the Photon Correlation Spectroscopy (PCS) machine and electrophoretic mobility titration (NanoZS4700 nanoseries, Malvern Instruments, Malvern, UK). All liposome formulations were diluted with 1 ml of filtered distilled water to eliminate the effect of viscosity caused by the ingredients. Hydrodynamic diameter (based on volume measurement), PDI and zeta potential were obtained from the average of three measurements at 25 °C. The refractive index of liposome and water were set at 1.42 and 1.33, respectively.

Atomic force microscope (AFM) imaging was conducted using a non-contact mode™ AFM (SPA400, Seiko, Japan). Liposomes were placed on a freshly cleaved mica surface, dried with a stream of nitrogen, and further dried in an electronic dry cabinet at 25 °C for 3 h. Experiments were performed with a tapping mode using NSG 10 cantilever at resonance frequencies of 190–325 KHz and a constant force of 5.5–22.5 N/m. All images were recorded in air at room temperature at a scan speed of 0.8 Hz. The phase image and topology were used to determine morphology of the liposomes.

### 2.5. Determination of % curcumin entrapment efficiency and loading capacity

Purified liposome was mixed with 1% Triton X-100 and vortex-mixed. After incubation for 1 h at room temperature in dark condition, the curcumin was separated from liposome by ultracentrifugation at 80,000 rpm for 90 min at 25 °C using Beckman ultracentrifuge (Le 90, Beckman, Palo Alto, CA, USA) using a TI-100X rotor. The content of curcumin was determined by high-performance liquid chromatography (HPLC) (WaterE600 system, Milford, MA, USA). Stock solutions were prepared by dissolving individual standard compounds in Methanol. The samples were filtered using Phenex RC 0.45 mm membrane filter (Millipore, Germany) and injected in triplicate into the HPLC using Hypersil GOLD column (Thermo Fisher Scientific Inc, Waltham, USA). The isocratic condition using mobile phase composed of 43% (w/w) acetonitrile and 57% of 1% (w/w) acetic acid in water. The flow rate was 1.0 ml/min, the injection volume was 20 μl, and monitored at 428 nm.

The % entrapment efficiency (%EE) and % loading capacity was calculated by applying Eq. (1) and (2).

$$\%EE = \frac{\text{total amount of determined curcumin}}{\text{initial amount of curcumin loading}} \times 100 \quad (1)$$

$$\%LC = \frac{\text{total amount of determined curcumin}}{\text{total amount of dried liposome}} \times 100 \quad (2)$$

### 2.6. In vitro release study

The experiment was performed to evaluate the amount of curcumin released from liposome vesicles. One milliliter of liposome suspension was loaded in dialysis bag with a molecular weight cut off at 3500 Dalton, CelluSEP membrane (Membrane Filtration Products Inc., Seguin, TX, USA.) and immersed in 20 ml of receptor medium (PBS buffer pH 7.4, 30% methanol and 10% Tween20), which was accurately controlled under shaking condition of 150 rpm at 25 °C in shaking incubator (VS8480; VISION, South Korea). At fixed time intervals, 1 ml of the samples were withdrawn from the receptor phase and replaced with equal volumes of medium of PBS buffer. The amount of curcumin was determined by HPLC as described in Section 2.5. The measurements were performed in triplicate.