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Preparation of calebin-a liposomes and its antiproliferation in human cancer cells

Abstract

Calebin-A is a curcuminoid compound isolated from turmeric. Herein, we investigated the antiproliferative effect of calebin-A liposomes on cell growth compared with calebin-A. In this study, the calebin-A liposomes were successfully prepared by thin film-sonication method. The antiproliferative activity was evaluated in mouse neuroblastoma Neuro-2a cells and human SW480 colorectal tumor cells. Calebin-A liposomes (CAL) exhibited relatively uniform particle size distribution and perfectly stable during storage period of 3 weeks. The growth of SW480 and N2A cell could be inhibited by CAL. Liposomes could be an effective delivery system to enhance the Calebin-A bioactivity. The results have an important implication for using calebin-A liposomes for colon cancer prevention and therapeutics.

Keywords: calebin-A, liposome, *In vitro*, cell viability

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Abbreviations: CAL, calebin-a liposomes; EL, empty liposomes; ELISA, enzyme-linked immunosorbent assay; DMSO, dimethyl sulfoxide

Introduction

The rhizome of *Curcuma longa* L. has played a vital actor in the pharmaceutical and functional food industries, and is extensively utilized as a traditional herbal medicine in Eastern and Southeastern Asia.[1] More than 235 compounds from *C. longa* L. have been isolated and identified, and most of them are phenolic compounds and terpenoids.[2] The compound use in medicine could be attributed to its beneficial influences in reducing cancer and carcinogenesis growth. Curcuminoids, a group of diarylheptanoids in *C. longa* L., are confirmed to be major active constituents responsible for its biological functions. Recently, a novel curcuminoid, the natural compound Calebin-A has been obtained from *C. longa* L.[3] The compound structure has a ferulic acid ester bond and lacks the characteristic feature of curcuminoid compounds i.e. 1, 3- diketonic structure. In vitro studies showed that Calebin-A exhibited a strong ability in protecting cells from β-amyloid insult, especially PC12 rat pheocromocytoma and IMR-32 human neuroblastoma cells. Moreover, Calebin-A was documented to induce apoptosis in drug-resistant human gastric cancer cells. However, in our previous studies, we found that the bioactivity of Calebin-A was severely inhibited because the water solubility of the compound was poor (about 7.8μg/ml) and it caused recrystallization of the compound *in vivo*. The solubility and bioactivity can be improved by designing novel formulations allowing parenteral drug delivery.

It may significantly improve the potential clinical applications of Calebin-A that a nanoparticle-based drug carrier was designed to enhance the solubility of the bioactive component. In this direction, various methods have been developed to deliver the curcumin complexes. Among polymeric nanoparticle-based approaches, liposomes offer promising increased therapeutic performance of anticancer drugs by enhancing their bioavailability. Previous studies have also exhibited that incorporation of curcumin into membranes powerfully enhances its stability and has a significant impact on the efficacy of liposome-bound curcumin.[4] The purpose of this research was to prepare CAL by thin film-sonication method and anti-colon cancer proliferation of calebin-A.

Materials and methods

Materials

Calebin-A (>97% pure) (Figure 1) was provided by Sabinsa Corp. (East Windsor, NJ, USA). L-α-phosphatidylcholine from egg yolk (EPC) and Tween 80 were purchased from Sigma Aldrich (Germany). Cholesterol (CHOL) used was from ACROS organics (Belgium). Chloroform was obtained from Mallinckrodt Chemicals (Ireland). PBS buffer (0.05M, pH7.0) was composed of sodium dihydrogen phosphate (Merck, USA) and disodium hydrogen phosphate (Wako Pure Chemical Industries, Ltd., Japan).

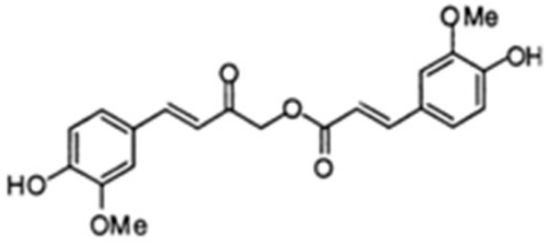

Figure 1 The chemical structure of Calebin-A.

Methods

Preparation of calebin-A liposomes (CAL)

CAL was prepared by thin film-sonication method.[5] Briefly, EPC (400mg), cholesterol (50mg), and Calebin-A (20mg) were dissolved in 20ml chloroform in a 250mL pyriform flask. The flask was rotated to evaporate the organic solvent using a rotary evaporator (Buchi RE11 Rotavapor, Switzerland) at 50oC, and the solution should avoid bubbling, and a thin film was formed on the internal wall of the flask. The thin film was further evaporated to expel the residual chloroform for 15min. And then 15ml PBS buffer containing Tween 80 200mg

was added in the flask. The flask was sequentially rotated to hydrate the thin film at 50°C from 30min, and a suspension was formed. The suspension was then sonicated under 200W for 5min (Ultrasonic Cleaner DC200H), and CAL was obtained.

Measurement of the particle size and zeta potential of calebin-A liposomes

The particle size of CAL was measured by DLLS technology using a particle size analyzer (90plus/BI-MAS, Brookhaven Instruments Corp.) at 25°C. The concentration of CAL was diluted to 0.1% (W/V) using PBS buffer solution. Each sample was measured and repeated three times. The zeta potentials of liposome samples were determined based on laser Doppler electrophoretic mobility theory using a zeta potential analyzer (90plus/BI-MAS, Brookhaven Instruments Corp.) at 25°C. The concentration of liposomes was diluted to 0.1% (W/V) by 0.05M PBS buffer (pH 7.0).

MTT assay

Neuro 2A (N2A) and SW480 cells were subculture into 96-well culture plates at a density of 105/well in 100μL of MEM and DMEM medium. The next day, the various concentrations (14-140μM) of empty liposomes (EL) and CAL were added. After 24h of incubation, the medium was discarded and cells were washed with PBS. 100μL of 0.2% MTT was added to each well, and the plates incubated at 37oC for 2 h. Then, 100μL of DMSO was added to each well and detected the absorbance of the oxidized MTT solution at 570nm by an ELISA reader. All experiments were performed in triplicate, and the mean value and standard error for each treatment were determined, then converted to percent relative to control (EL treatment).

Results

Photography of CAL

The photography of CAL suspension was shown in Figure 2. As can been seen from Figure 2, the CAL and EL suspensions were homogeneous. The EL suspension was pale yellow, while the color of CAL was deep yellow. CAL and EL suspensions were still very uniform after storage at 4oC for 3 weeks, and no floccules or precipitation was found in samples. In addition, the color of samples was not been changed after storage.

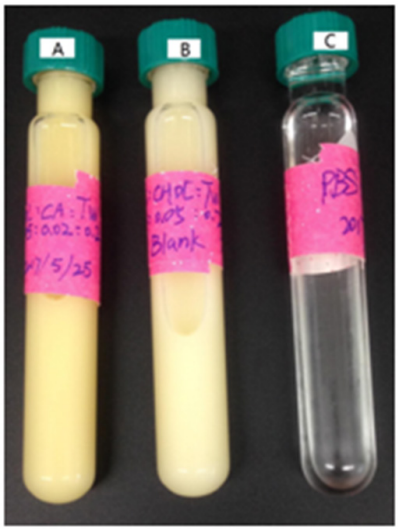

Figure 2 The photography of CAL suspension.

A: CAL Suspension; B: EL Suspension; C: PBS Buffer.

Particle size and zeta potential of CAL

Particle size is a key indicator for the bioavailability and bioactivity of liposomes. The particle size distribution of CAL and EL was showed in Figure 3. The average diameters of EL and CAL were 237.1nm and 555.5nm, respectively, while the polydispersity indexes of the two samples were 0.291 and 0.339, respectively. As shown in Figure 3, the particle sizes of CAL were distributed in a wider range compared with those of EL. Zeta potential is a critical and useful indicator of particle surface charge, which could be utilized to adjust the storage stability of suspension systems.[6] The zeta potentials of blank and CAL suspensions were -33.29 mV and -35.55 mV, respectively.

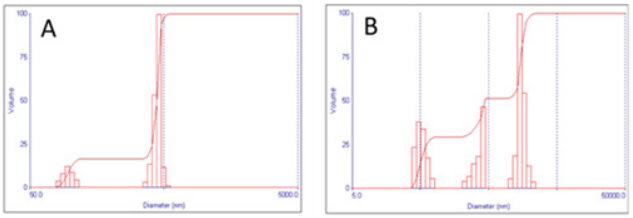

Figure 3 The particle size distribution of CAL and EL. A: EL; B: CAL.

MTT assay

The effects of CAL on the growth of SW480 and N2A cancer cells were assessed using MTT assay as previously described. Exposure to CAL for 24 hours inhibited SW480 and N2A cell growth in a concentration-dependent manner (Figure 4). Furthermore, compared with N2A cells, the cytotoxic effect of CAL was more pronounced in SW480 cells (Figure 4).

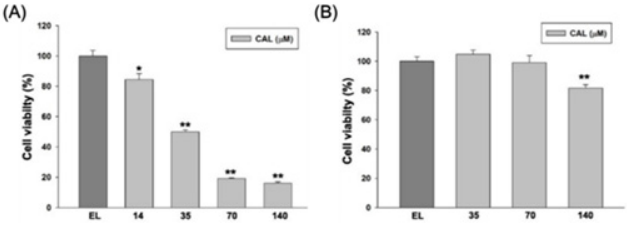

Figure 4 Effects of CAL on tumor cell viability.

(A) SW480 and (B) N2A cells were treated with EL and CAL for 24h. Cell viability was determined by MTT assay. The percentage of cell viability was calculated as the ratio (A570) of treated cells to control cells. Data represent the ±SE of three independent experiments. Statistical significance, *p< 0.05 and **p< 0.001 compared with the control group (EL treatment).

Discussion

It has been confirmed that Calebin-A is able to powerfully inhibit some cancer cell growth. However, the poor water solubility of the compound restrains its application. Nanoparticles have been considered to be an effective nanocarrier to deliver bioactive components. The solubility of core materials, i.e. the oil/water partition coefficient, plays a critical role in distributing in biological membranes.[6] It also was reported that the encapsulation efficiency of liposomes correlated to core material solubility. Generally speaking, the core material with high solubility in chloroform or in water could be easily encapsulated into lipid microvesicles.[7] Calebin-A could be easily dissolved in chloroform, so it could be speculated that the compound could be effectively encapsulated into liposomes. The color of drug-containing liposome was deepened after encapsulating the compound into liposomal vesicles. It suggested that Calebin-A could be stably encapsulated into liposomes. Meanwhile, the solubility

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of Calebin-A in water was effectively enhanced. In addition, due to the high absolute values of zeta potential of CAL and EL, a strong electrostatic repulsive force occurred among liposome particles.[8] It was beneficial to the stability of CAL suspension during storage period.

Due to the poor aqueous solubility of Calebin-A, the compound is usually dissolved in dimethyl sulphoxide (DMSO) to use.[1,3] More noteworthy was the fact that the antiproliferative effect of calebin-A in HCT116 cells was no significantly difference at 10 to 50μM concentrations.[3]. However, the results of recent experiments exhibited that CAL could effectively inhibit the growth of SW480 cells (IC50≈35μM), and especially the changes were associated with concentration-dependent manner. Thus, encapsulating in liposomes is a more safe method to change the solubility of calebin-A when compared with dissolved in DMSO. The fact that CAL could effectively inhibit the growth of SW480 cells could be mainly attributed to two reasons. First, the increase of Calebin-A solubility could enhance the effective concentration of the compound. Second, more than as a nano delivery system, liposomes also could alter the absorption pathway of the core material. Furthermore, it had proved that the delivery system also could change signaling process essential for basic cell functions and played a simulative role in mediating biological effects.[9] Herein, we first suggested that CAL exhibit promising potential to emerge as alternative therapeutics agent for colon cancer.

Conclusion

This study focused on the preparation and the in vitro evaluation of CAL. These data illustrated that Calebin-A could be successfully encapsulated into lipid microvesicles. The liposomes exhibited adequately stable. The CAL markedly inhibited SW480 cells proliferation than N2A cells. CAL will be further characterized and the bioactivity of the liposomes will be estimated in the future work, respectively.

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Conflicts of interest

Author declares there are no conflicts of interest.

Funding

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