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EARLY EXPERIMENTAL RESULTS OF COLORECTAL CARCINOMA CHEMOTHERAPEUTICS BY LIPOSOMAL CURCUMINOIDS

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Curcuminoids had shown anti-proliferation and induce the apoptosis in many cancer cells. It was chosen as the model drug and liposome, a biocompatible and biodegradable carrier was used (liposomal curcuminoids; LPC). The technique was also investigated for the possibility of facilitating the anti-tumor activity of LPC in colorectal cancer (CRC). *In-vitro* permeation studies in fresh pig colon mucosa; we found that reduced the cumulative amount and flux of LPC. There are 7.8 folds decreasing which compared to the curcuminoids treatment without liposomal encapsulation. Summary the results show that the liposomal encapsulation could enhance and prolong the cytotoxicity of curcuminoids through possibly increase the cellular uptake of curcuminoids in colorectal cancer cell (CRC) lines (CP1 to CP5). Through the use of LPC, the transmucosa drug delivery system (DDS) of liposome encapsulation to the CRC cancer therapy could be made possible.

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Keywords: Curcuminoids, colorectal cancer, Liposome

1. Introduction

Turmeric (Curcuminoid), an herb isolated from the rhizome of the *Curcuma Longa*. It has been a popular spice and contained in the Oriental food additives, like curry. It has been used extensively in the Asian countries, and also in the traditional medicine [1]. Interest in curcuminoids has grown in recent years because of its putative beneficial health effects, including antioxidant [2], anti-inflammatory [3] and cancer chemopreventive actions [4]. Studying the inhibition of cell proliferation and the induction of apoptosis by curcuminoids could achieve a better insight into the mechanisms underlying the cancer chemoprevention by curcuminoids.

Most chemotherapeutic agents have poor solubility and low bioavailability, and are formulated with toxic solvents [5-6]. Thus, the use of nano-carriers DDS allow for the preparation of low water soluble cancer drugs in liquid [6] like curcuminoids. Nano-medicine is an emerging field that deals with interactions between molecules, cells and drugs such as molecular. The National Nanotechnology Initiative (NNI) defines the nano- in dimensions of roughly 1 to 100 nm, but in boarder range $\leq$ 1 μm [7]. The nano-DDS size scale within this range appear to be optimal

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for achieving many important factors as nano-medicine, including the alteration of a drug's bioavailability, strength, electrical properties, and ultimately, its behavior *in vivo* and *in vitro* [8].

Liposomes are small lipid vesicles within the range of 50 to 1000 nm and have been studied extensively as drug carriers particularly for cancer therapy [9]. It's unique advantages over traditional drug, including their ability to protect the drugs from degradation, site-targeting for drug and minimizing the toxicity and side effects [10]. In other hands, the major problems associated with liposome are their stability, poor batch to batch reproducibility, difficulty in sterilization, and low drug loading capacity [11].

Using liposome in drug design and delivery, we are trying to push liposome to be able to deliver the curcuminoids to the targeted cancer cells, release the curcuminoids at a controlled rate, be a biodegradable DDS, and to be able to escape from degradation processes. In this report, studies have been investigated whether LPC could contribute to the anti-proliferation as well as the apoptosis of colorectal cancer cell lines (CP1 to CP5). The aims are to investigate whether the anti-proliferation and apoptosis activities of LPC on the CRC cell lines, using the cell lines from primary human adenocarcinoma (CP-1, -2, -3 and -5) or tubulovillous adenoma (CP-4) on the colon (2 cases), sigmoid colon (2 cases) or descending colon (one case) carcinoma could be explained, qualitatively and also quantitatively, by the bioavailability of LPC.

2. Experimental

2.1. Materials

Commercial curcuminoids (>97% Pure curcumin), which was selected as a model for drug was purchased from Merck KGaA (Germany). Double distilled water was prepared in house and was used to prepare the aqueous solutions. All products were used as received. All other chemicals were of reagent grade. The formulation of curcuminoids encapsulated liposome was list in Table 1.

2.2. Liposomal Formulation of curcuminoids

The liposome encapsulated curcuminoids were prepared by film hydration vesicle method as previously reported [12]. Liposomes were prepared using different ratios of 1,2-Dimyristoyl-sn-glycero-3-phosphocholine（DMPC）, Cholesterol（CH）, and curcuminoids. The particles were formed using probe sonication according to previously described procedures [13]. Briefly, a proprietary mixture of lipids (including DMPC, CH and curcuminoids) is dissolved in a chloroform/methanol solution (1:1, v/v). Thin lipid films are created by pipetting aliquots of the lipid solutions into round-bottomed glass tubes and evaporating the solvent at 37-40℃ under a stream of nitrogen gas. The films are placed under vacuum for at least 12 h to remove residual organic solvent. Liposomes are prepared by hydrating the lipid films with curcuminoids dissolved in citrate-phosphate buffer and then incubating the suspension at 65℃ for 30–40 min followed by probe sonication (25W, 10 min) until the suspension is translucent. In the resultant suspension, unencapsulated curcuminoids is removed by ultra-centrifuation (48000Xg 60 min, 4℃)(HITACHI himac CS 150 GXL) and the suspension is sterilized prior to use by passage through a 0.22 mm filter. The encapsulation ratio (ER) is analyzed by the equation (ER(%)=(Q1-Q2)/Q1x100), Q_1 : The theoretical amount of curcuminoids sample that was added Q_2 : The amount of curcuminoids samples were detected only in the supernatant. The curcuminoids concentration is determined by HPLC methods [14].

2.3. Characterization of LPC

Droplet size distribution and zeta potential of the LPC was determined by photon correlation spectroscopy (PCS) using a Zetasizer Nano-ZS (Malvern Instruments, UK). Particle solution (1.0 ml) was pipetted into a cuvette. Data were collected at room temperature (23-25℃). Zeta potentials of LPC were also analyzed by Zetasizer Nano-ZS (Malvern Instruments, UK). Each assay was carried out at least triplicate and the results were expressed as the mean+/-SEM.

2.4. Cell lines and culture conditions.

The specimens of human colon cancer were obtained from a total of five colon patients suffering from adenocarcinoma (CP-1, -2, -3 and -5) or tubulovillous adenoma (CP-4) on the colon (2 cases), sigmoid colon (2 cases) or descending colon (one case). These patients consisted of three men (age: 54-82 years) and two women (age: 62-72 years), who were receiving treatment in the Kaohsiung Medical University Hospital (KMUH). The specimens were taken during the operation for colon cancer removal, in the period of 1/13/04 to 5/10/05. Specimens were removed from only the typical and clinically clear-cut (Grade II) cases [14]. Prior written informed consent was obtained from the patients and all procedures used had been reviewed and approved by the ethics board at KMUH in adherence to the Helsinki Principles. The five primary cell lines of colon cancer cells were derived, as a gift, from the cell bank maintained in the MedicoGenomics Research Center at KMU [14]. The cells were grown at 37℃ in the Dulbecco's Modified Eagle Medium (GibcoBRL) supplemented with 10 % (v/v) Fetal Bovine Serum (HyClone) and a combination of antibiotics (penicillin, 200 unit/ml, and streptomycin, 200 g/mL) (HyClone) under an atmosphere CO_2/air (5%) for this series of studies.

2.5. Assay of cell proliferation.

The proliferation of the CRC cell lines after exposure to curcuminoids (Cur), LPC or liposome co-treated curcuminoids (LCC) was measured by the CellTiter96 Aqueous one solution cell proliferation assay kit (Promega, USA). Briefly, the cells were first incubated with curcuminoids for 24 hours and then treated with MTS [3-(4,5-dimethyl-thiazol-2-yl)-5-(3-carboxymeth- oxy-phenyl)-2-(4-sulfo-phenyl)-2Htetrazolium] for 4 hrs. The absorbance was determined by the Powerwave XS reader (Bio-Tek, USA) at 490nm. Each assay was carried out in triplicate and the results were expressed as the mean (+/-SEM). Cell proliferation was expressed as the percentage of the assay data determined for the control group.

2.6. Preparation of extracting solution

For achieving the efficient recovery of curcuminoids in the CRC cell lines, an extracting solution was developed. It was prepared by combining ethyl acetate with isopropanol, at a ratio of 9:1 (v/v) and the solution was stored at room temperature until uses (which was found stable for at least one month).

2.7. Recovery of curcuminoids from CRC cells for assay

The cell pellets were each suspended in the RIPA buffer [20mM of Tris/HCl (at pH 8.0), 5 mM of EDTA, 1mM of phenylmethyl-sulfonyl fluoride (PMSF), 1.5mg of leupeptin, 137mM of NaCl, 10 % of glycerol, and protease inhibitor cocktail], and the cell-liquid extraction was then carried out immediately. The cell extracts were centrifuged at 18,000 rpm for 15 min, the amount of total cellular proteins in the extracts was each determined by the Bradford method (Bio-Rad, USA). The cell extract obtained was acidified, 100µ each, by a 6N HCl (1:1 w/v) and vortexed for 30 seconds. Another volume of extracting solution (500 µL) was added to each of the acidified cell extracts, and samples were vortex again and then shaken in the Orbited shaker (at 100rpm) for 15min. After centrifugation at 18,000 rpm for 20 min, the upper organic layer was filtered by a membrane filter (0.22 µm) and transferred to a clean injection sample vial (~100 µL) for a quantitative analysis by the HPLC methods [14-15].

2.8. Evaluation of Apoptosis

The apoptosis was assessed by the ApopNexin FITC apoptosis detection kit (Chemicon, USA) and APOPercentage Apoptosis Assay kit (Biocolor Ltd. United Kingdom). Briefly, the primary colon cancer cells (5×10^3) were seeded in the culture medium containing 100 µL of serum and dispensed into the 96-well microplates. After treated with curcuminoids for 24 hours, the culture medium was replaced with a fresh medium containing the APOPercentage Dye Label. The change in cell morphology was determined microscopically by Olympus CKX41. The APOP% Dye Release Reagent was added to each well to aid cell lysis and the release of bound dye

from the apoptotic cells. The cells were treated with LPC for 6 hours and the apoptotic cells were detected by ApopNexin FITC apoptosis detection kit by Fluorescence Microscopy (Olympus CKX41 and U-RFLT 50) and flow cytometry (Facscalibur, BD) and data analyzed by WinMDI 2.8 free software (BD, USA)

2.9. Transmission Electron Microscope (TEM) and Confocal microscopy

LPC were processed for TEM using established protocols. Sections for TEM were visualized using a transmission electron microscope (model JEM 2000EX, Jeol, Japan). The liposome uptake in CRC cells was observed by confocal microscopy. The cells (2×10^4 cells/well) were seeded on cover glass in a 24-well tissue culture plate for 24 h after which the medium was removed, and 1.2 mL Earle's Balance Salt Solution (EBSS) was placed in each well. The orange-green 488 DSPE fluorescein labeled liposome in a volume of 300 μL were added per well to a total volume of 1.5 mL/well. After incubation for 1.5 h at 37 °C, the EBSS was removed, and 1.0 mL of fresh complete medium was added and the cells incubated for an additional 1 hour. The medium was then removed, the cells washed twice with PBS, fixed with 4% paraformaldehyde for 15 min, and washed twice with PBS. Subsequently, the Rhodamine 123 solution (Sigma-Aldrich, USA) was added, and incubated at room temperature for 30 min. The cells were again washed twice with PBS. The cover glasses were mounted using the Prolong Antifade Kit (Molecular Probes Invitrogen, Eugene, OR, USA) and the samples were imaged with laser scanning confocal microscope (BD^{TM} CARV II).

2.10. The colorectal mucosa permeation kinetics of LPC

The permeation profile of curcuminoids and LPC across pig colon mucosa and the effect of liposomal encapsulation for 24 hours. The use of liposomal encapsulation has detected the stability of curcuminoids during the course of Tranmucosal permeation by Villa-Chen cells and HPLC [14]. Each data point represents the mean ± SEM of triplicate studies (n=3)

2.11. Statistical analysis

All data are reported as the means (±SEM) of at least three separate experiments. Statistical analysis was used the *t* test, with the significant differences determined at the level of $P<0.05$.

3. Results

3.1. Effects of curcuminoids on the size and encapsulation efficiency in liposome formulations

Particular size of bare liposome (F1) and curcuminoids loaded liposomes (LPC: F2 to F5) were measured (Table 1). The average size of bare liposome and curcuminoids loaded liposome were 128+/-2.55 nm (F1), 147+/-3.56 nm (F2), 149+/-4.95 nm (F3), 175+/-1.52 nm (F4) and 119+/-0.71 nm (F5), respectively. The size of LPC (F2 to F4) was enlarged than bare liposome (F1), but the particular size still under 200nm. Nanoscale devices smaller than 50 nanometers can easily enter most cells, while those smaller than 20 nanometers can transit out of blood vessels.[16] However, anything smaller than 200nm is no longer absorbed by phagocytes thus nanoparticles can travel though the blood and move randomly throughout the entire body [17]. The formulation F3, F4 and F5 of curcuminoids loaded liposomes with well particular size (175nm~119nm) and encapsulation efficiency (99.46~86.37), but both of those formulations has worse hydration. In this study, bare liposome and curcuminoids loaded liposomes procedure as described in the Methods section and the liposome (F2) was visualized using a transmission electron microscope (Figure 3 A).

3.2. Liposome formulation could prevented the degradation of curcuminoids by light and pH 7.4 buffer system

The curcuminoids could inhibit cancer cell proliferation and the induction of apoptosis in cancer cells may via the antioxidant activity. The light or pH 7.4 buffers will destroy antioxidant

structure and reduce the bioactivity. The liposome formulation significantly reduced the degradation of curcuminoids by light exposition (Figure 1. A) and pH 7.4 (Figure 1. B).

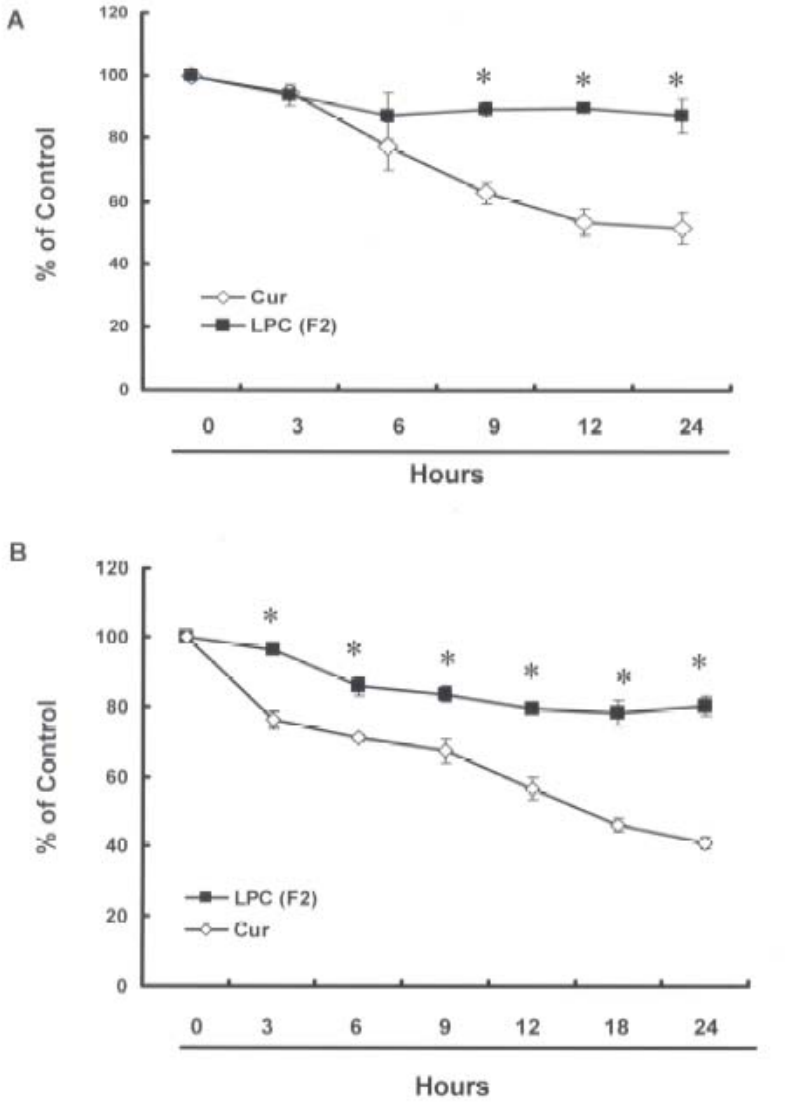

*Fig. 1. Comparative degradation profile of curcuminoids with liposomal encapsulation. The sample with or without (A) light exposure (B) pH 7.4 buffer for 24 hours. Each data point represents the mean ± SD. Statistical analysis used the t-test, with the significant differences determined at the level of *P<0.05 versus solution group.*

3.3. Effects of preserved temperature and time on the particle size and zeta potential in liposome formulation.

To explore whether the effects of preserved temperature (4, 25 and 37℃) and time in LPC (F2), the particular size (Figure 2.A) and zeta potential (Figure 2.B) has been determined in 1, 2, 3 and 4 weeks. The results summarized in Figure 2A indicate that the particular size in 4 and 25 ℃ has no significant different compared to control (0 day). Figure 2.B shown, the zeta potential of F2 will reduced along with time, but the 4℃ is better preserved temperature for liposome storage. Therefore the samples of this study preserved in 4 degrees C, and uses in one month.

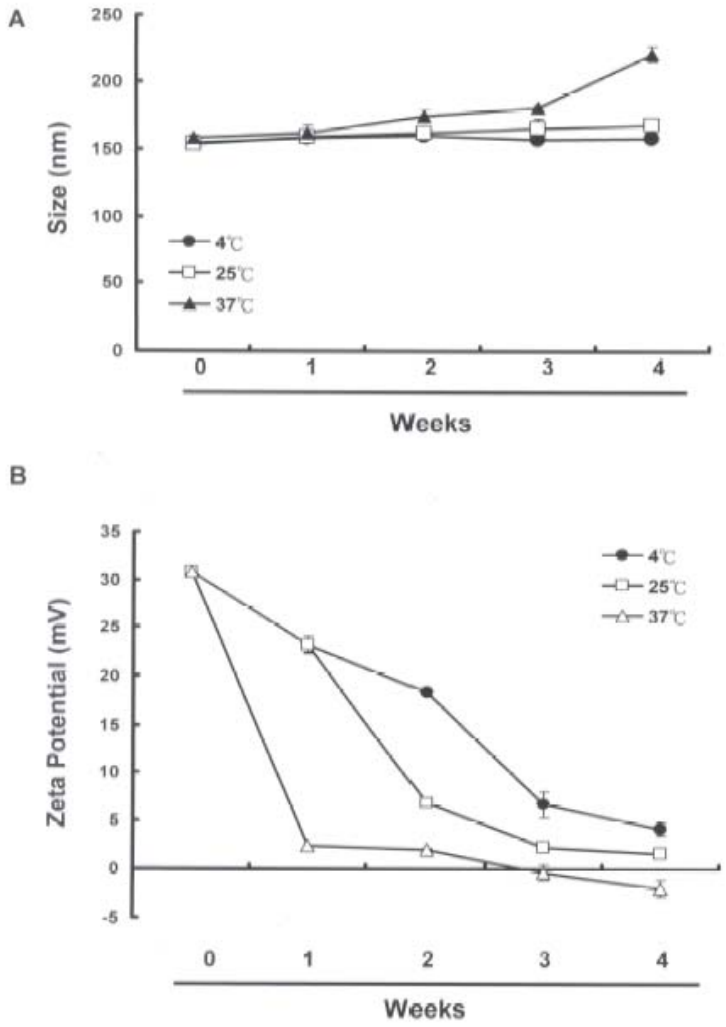

Fig. 2. Comparative degradation profile of liposome (F2). The particular size (A) and zeta potential (B) has been determined by Zetasizer Nano-ZS (Malvern Instruments, UK) in 1, 2, 3 and 4 weeks.

3.4. Proliferation of CRC cell lines were inhibited by LPC

In this series of studies, a hypothesis has been proposed, that is: LPC could mediate the survival of CRC cancer cell lines and thus should inhibit their proliferation (Figure 3.B). To validate this hypothesis as well as exploring the anti-tumor activity of LPC against the breast cancer cells, an in vitro study was initiated and each of these five human primary CRC cancer cell lines (CP1 to CP5) was treated with an increasing dose of curcuminoids in F2 (0, 2.5, 5 and 7.5 µg/ml) for 24 to 72 hours. The proliferation of this LPC (F2) -treated cancer cells was then each assayed by MTS test. The results summarized in Figure 3.B indicate that the survival and proliferation of CRC cancer cells have been reduced as increasing the dose of curcuminoids used in the treatment. The survival and proliferation of CRC cancer cells have shown a dose-dependent reduction in all five cell lines. Moreover, the IC_{50} of CRC cell lines were found to vary with the type of CRC cancer cells (Table 2), that is: The CRC cancer cell lines is more sensitive to the anti-proliferative action of LPC than Cur (Curcuminoids only) and LCC (bare liposome co-treated curcuminoids) in the 24-hr exposure.

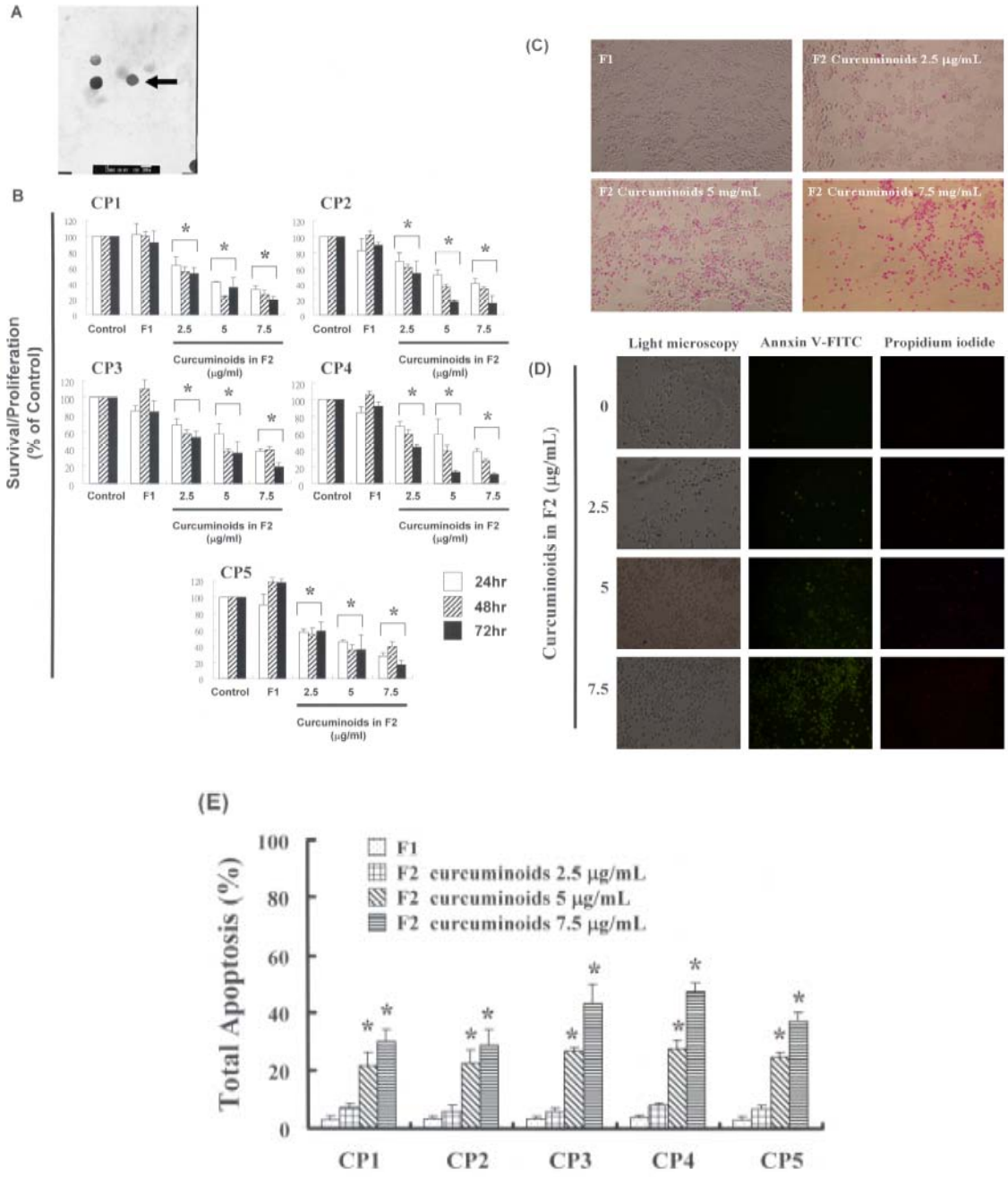

*Fig. 3. (A) LPC (F2) were visualized using a transmission electron microscope (model JEM 2000EX, Jeol, Japan). (B) LPC mediates the survival of five colorectal cancer cell lines (CP1, CP2, CP3, CP4 and CP5) and thus inhibits their proliferation. Each data point represents the mean ± SD. Statistical analysis used the t-test, with the significant differences determined at the level of *P<0.05 versus F1 group. The LPC induces apoptosis in CRC cancer cell lines (C) the cells with different doses curcuminoids (2.5, 5.0 and 7.5 μg/mL) of LPC, the percentage of apoptotic cells were assessed by APOPercentage Apoptosis Assay kit (C) and Annexin V-FITC and propidium iodide staining (D), followed by Fluorescence Microscopy and flow cytometric analysis. (E) Total apoptosis (early and late) analysis of five CRC cancer cell lines after culturing with LPC. All data were reported as the means (±SEM) of at least three separate experiments. Statistical analysis used the t-test, with the significant differences determined at the level of *P<0.05 versus F1 group.*

3.5. Induction of Apoptosis-Dependent Cell Death by LPC in CRC cancer cell lines

To further elucidate the anti-cancer mechanism of LPC in CRC cancer cell lines, we performed apoptosis studies. After treating the cells with different doses curcuminoids (2.5, 5.0 and 7.5 μg/mL) of LPC F2, the percentage of apoptotic cells were assessed by APOPercentage Apoptosis Assay kit and Annexin V-FITC and propidium iodide staining, followed by flow cytometric analysis.

A typical set of results for APOPercentage Apoptosis assay is illustrated in Figure. 3(C), in which the pink-colored deposits are indicative of the positive existence of apoptotic cells. A dose-dependent increase in apoptosis was observed, that is: the higher the dose of curcuminoids in F2 (0, 2.5, 5 and 7.5 μg/mL), the greater the extent of apoptosis. The Annexin V-FITC fluorescence versus PI fluorescence also indicated a significant increase of the percentage of apoptotic cells that were treated by the LPC F2. It was observed that at concentrations of 5.0 and 7.5 μg/mL curcuminoids loaded LPC, there was a significant increase in the percentage of cells undergoing apoptosis (Figure 3 D). Detection between the intact cells, early apoptotic cells and late apoptotic cells or dead cells could be carried out with PI-annexin-V double staining; thus, we performed this assay to further explore cell apoptosis. A dose-dependent increase in apoptosis was observed, that is: the higher the dose of curcuminoids in F2 (5 and 7.5 μg/mL) used in the exposure, the greater the extent of apoptosis (Figure. 3E). The increase of the percentages of apoptotic CRC cell lines was observed in all the doses after treatment for 6 h. In 6 h, approximately 3.21±0.3% of five CRC cells were total apoptotic (early apoptosis and late apoptosis) cells in control. The rate of apoptotic CRC cells increased to 6.72±0.84% with 2.5 μg/mL curcuminoids in F2 treatments. When the concentrations of curcuminoids increased to 5 and 7.5 μg/mL in F2, the percentages of total apoptotic CRC cells raised to 24.44±2.64% and 37.42±8.2%, respectively. Taking together; the observations have implied that significantly elevated the apoptosis of CRC cell lines by LPC (F2) treatment.

3.6. Assay of cellular and mucosa's curcuminoids form LPC release.

To characterize the kinetics of the uptake of curcuminoids in LPC by the CRC cancer cells, HPLC method has been developed in order to quantitate the level of curcuminoids taken up by the cancer cells after each treatment. Using the HPLC system and conditions developed and outlined above in the "Methods" section, the major components of curcuminoids: curcumin, the principal constituent, and its demethoxylated derivatives (dihydrocurcumin, or DMC and bisdemethoxycurcumin, or bDMC), were observed to yield three well-defined chromatographic peaks.[14] The results imply that curcumin and its demethoxylated derivatives (DMC and bDMC) could be assayed, quantitatively and reproducibly by the HPLC method in this study. It was observed that at concentrations of 5 and 7.5 μg/mL LPC, there was a significant increase in the cellular uptake in human CRC cancer cell lines (Data not shown). Confocal imaging was used to determine the cellular uptake of the LPC. Human CRC cancer cells (CP1) were incubated with Rhodamine 123 (red) and LPC was green fluorescein tagged. As shown in Figure 4A, the LPC delivery system transport uptake by tumor cells. By Orange-Green 488 DSPE labeled LPC, the results imply that the LPC was uptake in pig colon mucosa in vitro with Villa-Chien method (Fig. 4B) [14].

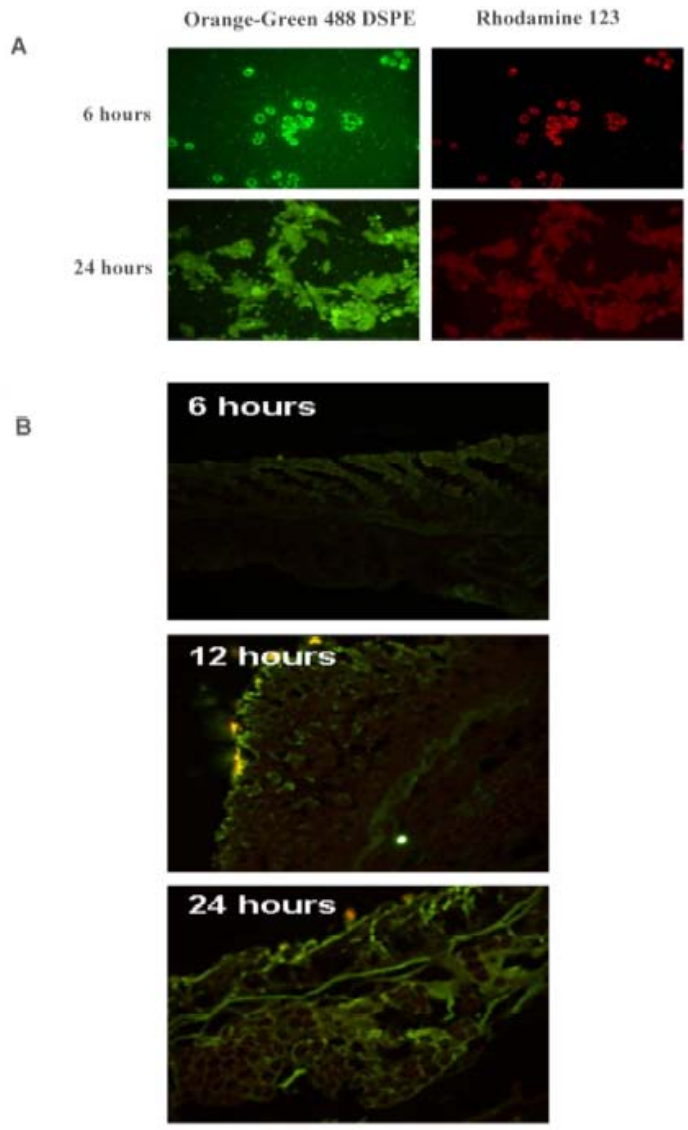

Fig. 4. Imaging of the (A) cellular (B) mucosa uptake by the LPC treatment in human colorectal cancer cells. The cells were incubated with Rhodamine 123 (red) and LPC was green fluorescein tagged for 6 and 24 hours. The image was by detected by Fluorescence Microscopy.

3.7. In-vitro mucosa permeation of LPC

The mucosa permeation studies in pig fresh colon mucosa, we found that LPC has lowered the cumulative amount and flux than curcuminoids only (Figure 5A). There are 7.8 folds increasing which compared to the curcuminoids without liposomal encapsulation (38.20 vs. 4.88 $\mu g/cm^2$). The LPC was a significant decrease in the cumulative permeation and Flux of curcuminoids in mucosa permeation (Table 3), but enhanced the mucosal partition profile of curcuminoids in 24 hours treatment (Figure 5B).

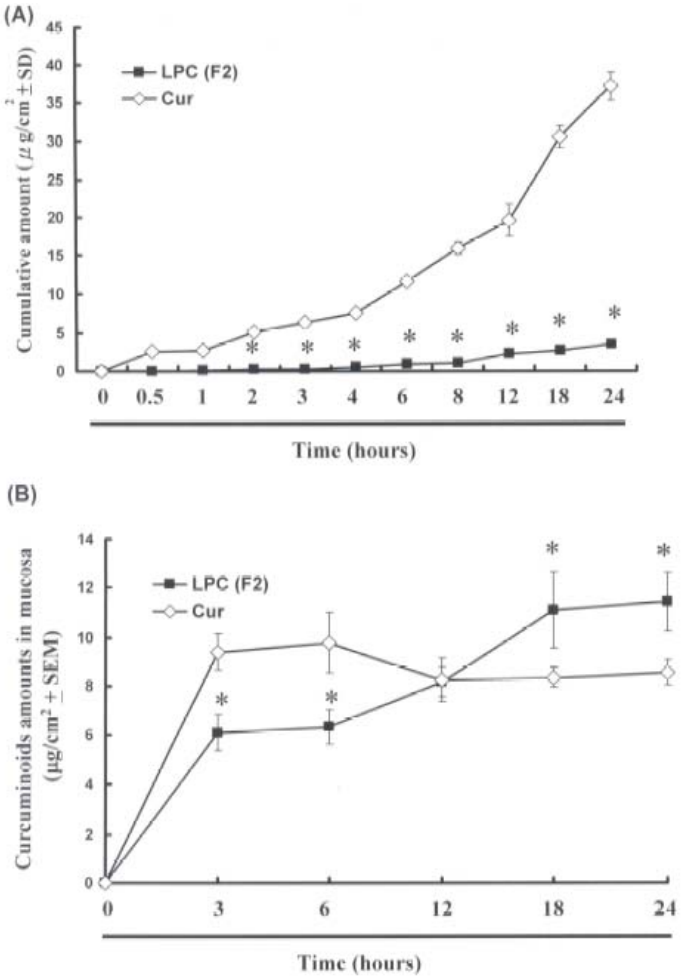

*Fig. 5. The permeation and mucosal partition profile of curcuminoids across pig colon mucosa. The effects of liposomal encapsulation for 24 hours in pig colon mucosa. Each data point represents the mean ± SEM of at least triplicate studies. Statistical analysis used the t-test, with the significant differences determined at the level of *P<0.05 versus Cur group.*

4. Discussion

Chemotherapy drugs are toxic to both tumor and normal cells, thus the efficacy of chemotherapy is often limited by the side-effects of the drug. Systemic administration of chemotherapeutic agents results in indiscriminate drug distribution and severe toxicity [18]. Nano-medicine has the power to radically change the way in cancer treatment. Now, there are many research going on to design novel nanocarrier capable of detecting cancer at its earliest stages [19], pinpointing it's location within the body and delivering antitumor drugs specifically to malignant cancers [20]. Nano-carriers are already proving that they can transport therapeutic agents to target cells and have the potential to radically change cancer therapy by increasing the number of highly effective therapeutic drugs [21]. This may allow for lower doses of toxic substances as the drugs are delivered directly to the target tissue. The liposomes can be targeted to cancer cells, it increase selectivity of drugs towards cancer cells and will reduce the toxicity to normal tissue and cells.

Curcuminoids was found to produce, in a number of cell types, an anti-tumor activity and induce apoptosis [22]. The results collected in this series of studies with the cell lines of colorectal carcinoma (isolated from the colorectal cancer patients) have provided experimental evidence to indicate that liposome encapsulated curcuminoids could irreversibly induce the apoptosis of CRC cancer cells.

Table 1. Formulations of curcuminoids encapsulated in liposome

Formulation	Curcuminoids (100μg/ml)	DMPC :CH (molar ratio)	Size (nm)	Encapsulation Efficiency
Cur	+	-	ND	ND
F1	-	1 : 1	128 ± 2.55	96.85 ± 0.12
F2	+	1 : 1	147 ± 3.56	97.1 ± 0.16
F3	+	2 : 1	149 ± 4.95	92.91 ± 0.35
F4	+	4 : 1	175 ± 1.52	99.46 ± 0.22
F5	+	8 : 1	119 ± 0.71	86.37 ± 1.51

DMPC: Dimyristoylphosphocholine
CH: Cholesterol
Cur: Curcuminoids free form

* P<0.05

Table 2. Effect of liposomal encapsulation on the cytotoxicity (IC_{50}) of curcuminoids in human colorectal cancer cell lines (CP1 to CP5).

Cell lines	Cur (μg/ml)	LPC (μg/ml)	LCC (μg/ml)
CP1	16.90±2.27	5.26±0.11 *#	17.11±1.32
CP2	17.75±1.37	7.68±0.59 *#	19.29±1.73
CP3	18.91±1.38	7.58±0.31 *#	20.16±1.26
CP4	19.86±2.23	6.34±0.38 *#	21.19±1.64
CP5	16.10±0.91	5.99±0.35 *#	19.33±1.77

LPC: Liposomal curcuminoids (F2)
LCC: Liposome co-treated curcuminoids
Cur:curcuminoids free form

* Vs. Cur P<0.05
# Vs. LCC P<0.05

Table 3. The pig colon mucosa permeation kinetics of curcuminoids and LPC

Formulation	Cumulative permeation in 24 hrs (μg/cm²±SD)	Flux (μg/cm²/hr±SD)
Curcuminoids in solution	38.20 ± 5.28	1.59 ± 0.22
Curcuminoids In liposome	4.88 ± 1.55 *	0.20 ± 0.06 *

Symbol (*) denotes that the difference of cumulative permeation in 24 hour permeation study, between curcuminoids solution and curcuminoids encapsulated in liposome. Symbol (*) denotes that the difference of flux in 24 hour permeation study, between curcuminoids solution and curcuminoids encapsulated in liposome, is statistically significant at $p < 0.05$.

Surface modification of nanocarrier (liposome) can also enhance the permeability of drugs to create high-permeability nanoparticle-based cancer therapeutics. To study the anti-tumor effects of liposome encapsulated curcuminoids in CRC cancer cells, five CRC cancer cell lines (CP1 to CP5) were selected. The cell viability of CRC cancer cell lines were found to decrease as elevated the loading level of curcuminoids in the liposome. The IC_{50} value of the LPC was found to be

lower than the non-encapsulated curcuminoids (Cur) in CRC cancer cell lines (Table 2). Those results suggest that the liposome enhanced the cellular uptake of curcumin into the CRC cancer cell lines. The liposomal encapsulation was also observed with a sustained anti-proliferation effect. In addition to, the HPLC technique was developed for quantization the mucosa uptake of curcuminoids (Table 3). The cellular uptake of curcuminoids was extracted form the CRC cancer cells and the results shown that a dose-dependent manner with the concentration of curcuminoids.

5. Conclusions

The cellular uptake of curcuminoids in the liposomal formulation was higher than free-form curcuminoids and the liposomal curcuminoids for transmucosal drug delivery system has potential for a wide range of CRC cancer research purposes and clinical applications.

Acknowledgments

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