Digest Journal of Nanomaterials and Biostructures Vol. 7, No. 1, January - March 2012, p. 59 - 71

LIPOSOMAL CURCUMINOIDS FOR TRANSDERMAL DELIVERY: IONTOPHORESIS POTENTIAL FOR BREAST CANCER CHEMOTHERAPEUTICS

CHI-CHANG CHANG[a], WEI-TE YANG[b], SHUN-YAO KO[c,d], YI-CHIANG HSU[c,d *]

[a]*Department of Obstetrics & Gynecology, E-Da Hospital, E-Da Hospital/I-Shou University, Kaohsiung, Taiwan.*

[b]*Graduate Institute of Pharmaceutical Sciences, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan.*

[c]*Graduate Institute of Medical Science, College of Health Sciences, Chang Jung Christian University, Tainan, Taiwan.*

[d]*Innovative Research Center of Medicine, College of Health Sciences, Chang Jung Christian University, Tainan, Taiwan.*

Curcuminoids derived from the rhizomes of *Curcuma longa* have demonstrated anti-proliferation and the ability to induce apoptosis in cancer cells. This study selected curcuminoids as the model drug and liposome as a biocompatible and biodegradable carrier (liposome encapsulated curcuminoids; LEC). The iontophoresis technique was also investigated for its potential in facilitating the skin permeation of LEC. *In-vitro* permeation studies using a newborn pig skin indicated that iontophoresis (ITP) elevates the accumulation and flux of LEC, with a five-fold increase over that of curcuminoids without liposomal encapsulation (7.52 vs. 1.60 μg/cm^2). Results demonstrate that liposomal encapsulation could enhance and prolong the cytotoxicity of curcuminoids through an increase in the cellular uptake and transdermal delivery of curcuminoids in breast cancer therapy. The use of ITP could enable the transdermal delivery of liposome encapsulation during breast cancer therapy.

(Received November 22, 2011; Accepted January 13, 2012)

*Keywords***:** Curcuminoids, Breast cancer, Liposome, Iontophoresis

1. Introduction

Turmeric (Curcuminoid) is a herb isolated from the rhizome of the plant *Curcuma Longa*. It is a popular spice in oriental food such as curry, and has been used in traditional medicine throughout Asia [1]. Interest in this herb has grown in recent years due to its putative health benefits, including antioxidizing [2], anti-inflammatory [3], and cancer chemopreventive activity [4]. A study of the inhibition of cell proliferation and the induction of apoptosis by curcuminoids could provide insight into the mechanisms underlying the cancer chemoprevention of curcuminoids.

Most chemotherapeutic agents are formulated with toxic solvents and show poor solubility and bioavailability [5]. Thus, the use of nanocarriers in the drug delivery system (DDS) allows for the preparation of low water soluble cancer drugs in solution [6]. Nano-medicine is an emerging field dealing with interactions between molecules, cells, and drugs. The National Nanotechnology Initiative (NNI) defines nano- as within dimensions of roughly 1 to 100 nm, but can include the broader range of ≤ 1 μm [7]. The scale of nano-DDS appears to be ideally suited to achieving many important objectives of nano-medicine, including the modification of a drug's bioavailability,

*Corresponding author: d8702008@tmu.edu.tw or jenway@mail.cjcu.edu.tw

strength, and electrical properties, and ultimately its behavior *in vivo* and *in vitro* [8].

Liposomes are small lipid vesicles in the range of 50 to 1000 nm, which have been studied extensively as drug carriers, particularly for cancer therapy [9]. The unique advantages of using Liposomes over traditional drug therapy include their ability to protect drugs from degradation, minimize toxicity and side effects, and facilitate site-targeting [10]. Conversely, the major problems associated with liposomes are their lack of stability, poor batch to batch reproducibility, difficulties in sterilization, and low drug loading capacity [11].

In the design and delivery of drugs, liposomes are induced to deliver curcuminoids to targeted cells and release them at a controlled rate, while providing a biodegradable drug delivery system capable of avoiding degradation through various processes in the body. In this report, we evaluate whether LEC could contribute to the anti-proliferation as well as to the apoptosis of breast cancer cell lines. The objective was to determine whether the anti-proliferation and apoptosis activity of LEC on breast cancer cells could be explained qualitatively and quantitatively (by the bioavailability of curcuminoids), using the cell lines from two human breast adenocarcinoma and one human ductal carcinoma. To achieve this end, we developed a sensitive and reproducible assay method, using high performance liquid chromatography, to quantitate the cellular uptake of curcuminoids by breast cancer cell lines.

2. Experimental

2.1. Materials

Commercial curcuminoids (> 95% pure curcumin) were purchased from Sigma-Aldrich (USA). Double distilled water used to prepare aqueous solutions was prepared in-house. All products were used as received. All other chemicals were of reagent grade. The formulation of curcuminoids encapsulated liposome is listed in Table 1.

2.2. Liposomal Formulation of curcuminoids

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

2.3. Characterization of LEC

The droplet size distribution and zeta potential of the LEC 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℃). The zeta potentials of the curcuminoids loaded liposomes were also analyzed with a Zetasizer Nano-ZS (Malvern Instruments, UK). Each assay was performed in at least triplicate, and the results were expressed as the mean (+/- SEM).

2.4. Cell lines and culture conditions

The cell lines used in this investigation: MDA-MB-435S (human ductal carcinoma), MDA-MB-231 (human breast adenocarcinoma) and MCF-7 (human breast adenocarcinoma), were obtained from ATCC (Manassa, Virginia, USA) and maintained in culture medium (GibcoBRL)

according to the manufacturer's specifications. The medium was supplemented with 10-15% (v/v) fetal bovine serum (HyClone) and a combination of antibiotics (penicillin, 200 unit/ml, and streptomycin, 200 g/ml) (HyClone) with or without an atmosphere of CO_2/air (5%).

2.5. Assay of cell proliferation

The proliferation of the breast cancer cell lines following exposure to curcuminoids (Cur), LEC or liposome co-treated curcuminoids (LCC) was measured using CellTiter96 Aqueous one solution cell proliferation assay kit (Promega). The cells were first incubated with curcuminoids for 24 h and then treated with MTS [3-(4,5-dimethyl-thiazol-2-yl)-5-(3-carboxymeth-oxy-phenyl)-2-(4-sulfo-phenyl)-2Htetrazolium] for 4 h. Absorbance was determined using the Powerwave XS reader (Bio-Tek) at 490 nm. Each assay was performed in triplicate and the results are expressed as the mean (+/-SEM). Cell proliferation is expressed as the percentage of the assay data determined for the control group.

2.6. Preparation of extracted solution

To efficiently recover curcuminoids from breast cancer cells, we developed an extraction solution by combining ethyl acetate with isopropanol, at a ratio of 9:1 (v/v). The solution was stored at room temperature until used (which was found to remain stable for at least one month).

2.7. Recovery of curcuminoids from breast cancer cells for assay

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

2.8. Recovery of curcuminoids from culture medium for assay.

To quantitate the curcuminoids remaining after the cellular uptake study, the residual amount of curcuminoid in the culture medium was assayed by first acidifying the culture medium (500 μL each) with 6N HCl, at the ratio of 1:1 (v/v), and vortexed for 30 seconds. Following the addition of the extraction solution (500μL) prepared above, samples were vortexed again and then shaken in an orbital shaker (at 100 rpm) for 15 min. Following centrifugation at 18,000 rpm for 20 min, the upper (organic) layer was filtered through a membrane filter (0.22 μm) and transferred to a clean injection sample vial (~100 μL) for quantitative analysis, according to the HPLC method described below.

2.9. HPLC analysis of curcuminoids

The HPLC system used to perform the HPLC analysis of curcuminoids was a Hitachi L-2000 series High Performance Liquid Chromatography system (Hitachi, Japan), comprising a solvent delivery system (L-2130 model) equipped with a fluorescence detector (L-2480 model), an autosampler (L-2130 model), and a 250×4 mm reversed-phase column (PurospherSTAR RP-18e, 5 μm, Merck) maintained at 37℃ by a column oven (L-2350 model). A mobile phase, comprising acetic acid (2%), at pH 2.5, and acetonitrile (at a ratio of 47:53) and delivered at a flow rate of 0.8 ml/min, was used, and the samples were injected with an injection volume of 20 μL. At an excitation wavelength of 420 nm and emission wavelength of 540 nm, curcumin, demethoxycurcumin (DMC), bisdemethoxycurcumin (bDMC) were detected [15].

2.10. Evaluation of Apoptosis

Following treatment with LEC for 6 h, apoptotic cells were detected using ApopNexin FITC apoptosis detection kit (Chemicon, USA) and flow cytometry (Facscalibur, BD) and data were analyzed using WinMDI 2.8 free software (BD, USA)

2.11. Transmission Electron Microscope (TEM) and Confocal microscopy

LECs were processed using TEM and confocal microscopy. TEM sections were visualized using a transmission electron microscope (model JEM 2000EX, Jeol, Japan). Liposome uptake in breast cancer cells was observed using confocal microscopy. Cells (2×104 cells/well) were seeded on a cover glass in a 24-well tissue culture plate for 24 h, after which the medium was removed and 1.2 mL of Earle's Balance Salt Solution (EBSS) was added to each well. Orange-green fluorescein labeled liposome (300 μL) was added to each well to a total volume of 1.5 mL/well. Following incubation for 1.5 h at 37 °C, EBSS was removed, 1.0 mL of fresh complete medium was added, and the cells were incubated for an additional hour. The medium was then removed, the cells washed twice with PBS, fixed with 4% paraformaldehyde for 15 min, and washed twice with PBS. Rhodamine 123 solution (Sigma-Aldrich, USA) was then added, followed by incubation at room temperature for 30 min. The cells were again washed twice with PBS. Cover glasses were mounted using the Prolong Antifade Kit (Molecular Probes Invitrogen, Eugene, OR, USA) and the samples were imaged using a laser scanning confocal microscope (BD^{TM} CARV II).

2.12. Skin permeation kinetics of LEC

A permeation profile of curcuminoids and LEC on a newborn pig skin and the effect of liposomal encapsulation and iontophoretic current (0.5mA/cm^2), applied at a pulse pattern (3-hr on & 3-hr off), for 24 h was determined. The use of liposomal encapsulation enhanced the electrostability of curcuminoids during the course of transdermal permeation. Each data point represents the mean (± SEM) of the triplicate measurements (n=3)

2.13. Statistical analysis

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

3. Results

3.1. Effects of curcuminoids on the size, polydispersity index (PI) and zeta potential in liposome formulations

We measured the particle size of bare liposome (F1) and curcuminoid loaded liposomes (Table 1). The average size of bare liposome and curcuminoid loaded liposome was 148+/-1.01 nm (F1), 163+/-1.05 nm (F2), 233+/-1.62 nm (F3), and 210+/-1.60 nm (F4). The size of curcuminoids loaded (F2) was 1.10 times larger than that of bare liposome (F1), but the particle size remained under 200 nm. Nanoscale devices smaller than 50 nm are capable of easily entering most cells, while those smaller than 20 nm can transit out of blood vessels [16]. However, anything smaller than 200 nm is no longer absorbed by phagocytes, thus nanoparticles are capable of traveling through the blood and moving randomly throughout the entire body [17]. The formulation of F3 and F4 curcuminoid loaded liposomes between 200 nm and 1000 nm could reduce this effect. The procedures using bare liposomes and curcuminoid loaded liposomes are described in the Methods section. Surface electrical potentials (Zeta potentials) for formulations F1 to F4 are shown in Table 1. Liposome F2 was visualized using a transmission electron microscope (Fig. 3 A). The average Zeta potentials of bare liposomes (F1) and curcuminoid loaded liposomes were 20.6+/-1.37 mV (F1), 21.4+/-1.56 mV (F2), 21.1+/-1.37 mV (F3), and 22.6+/-0.73 mV (F4).

Table 1. Formulations of curcuminoids encapsulated in liposome

Formulation	Curcuminoids (μg/ml)	DMPC :CH (molar ratio)	Stearylamine (1mg)	Size (nm)	Polydispersity index (PI)	Zeta potential (mV)
Cur	100	-	-	ND	ND	ND
F1	-	1 : 1	+	148±1.01	0.240±0.004	20.6±1.37
F2	100	1 : 1	+	163±1.05	0.250±0.003	21.4±1.56
F3	100	2 : 1	+	233±1.62 *	0.274±0.004	21.1±1.37
F4	100	1 : 2	+	210±1.60 *	0.355±0.027	22.6±0.73

DMPC:Dimyristoylphosphocholine
CH:Cholesterol
Cur:curcuminoids free form

(Mean±SD, N=3) * P<0.05

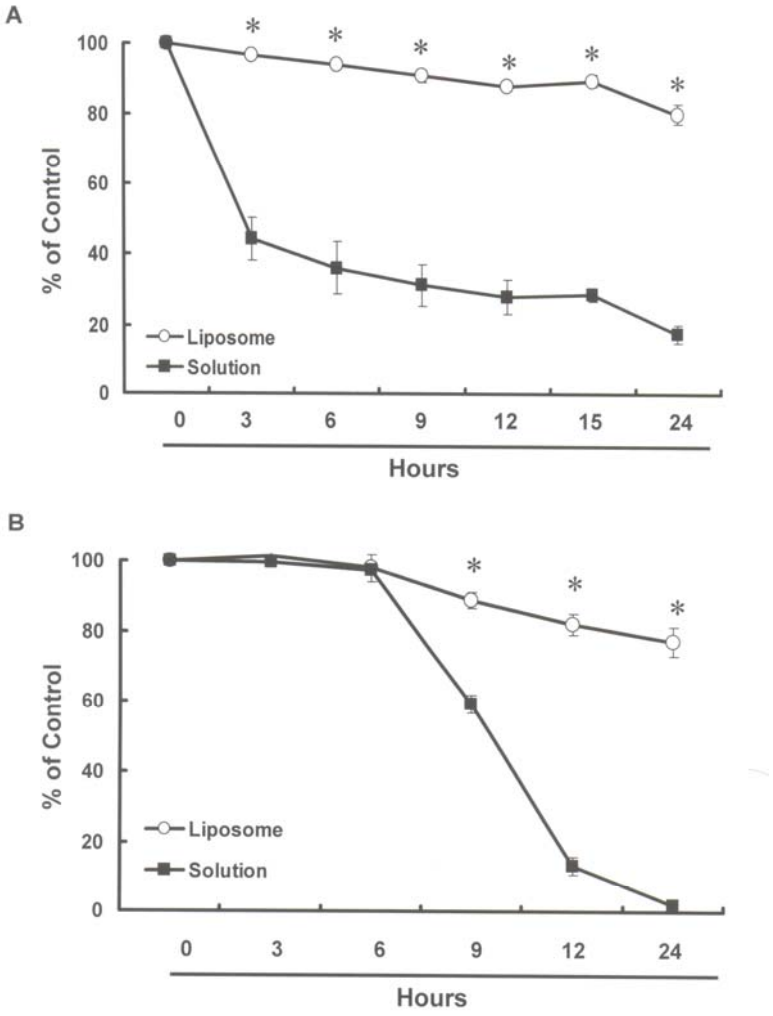

*Fig. 1. Comparative degradation profile of curcuminoids with liposomal encapsulation. Samples with or without (A) H_2O_2; (B) exposure to light for 24 h. Each data point represents the mean (±SEM). Statistical analysis used the t-test, with significant differences determined at a level of *P<0.05 versus the solution group.*

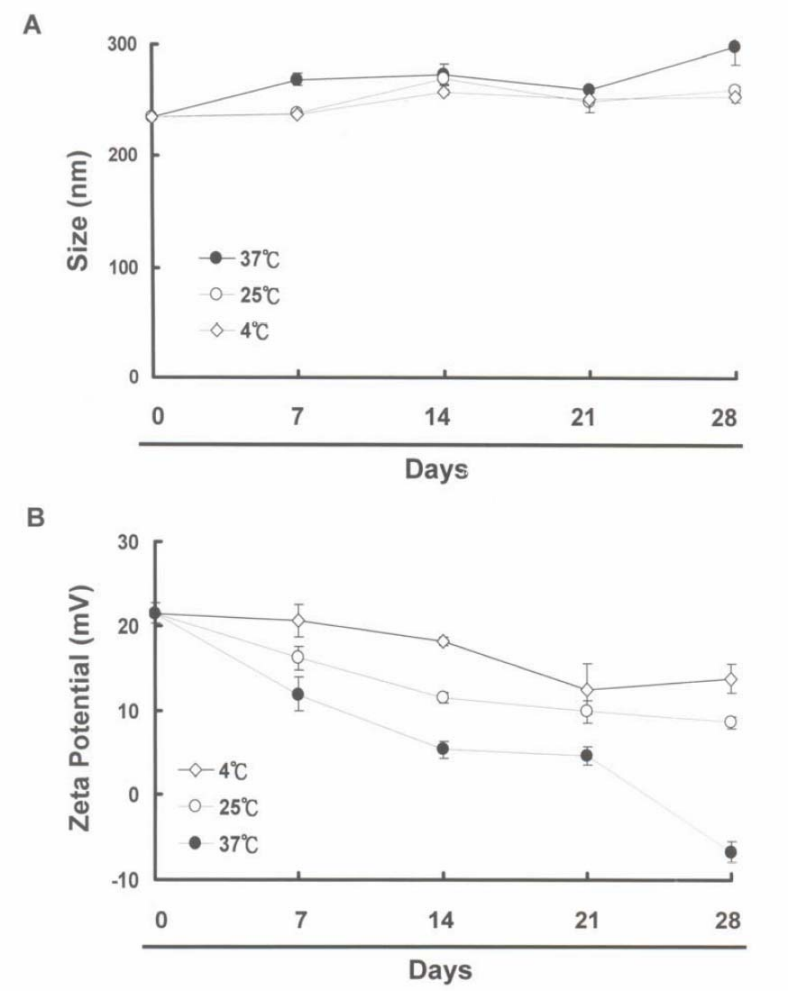

Fig. 2. Comparative degradation profile of liposome (F2). Particle size (A); and zeta potential (B) as determined by Zetasizer Nano-ZS (Malvern Instruments, UK) after 1, 2, 3, and 4 weeks.

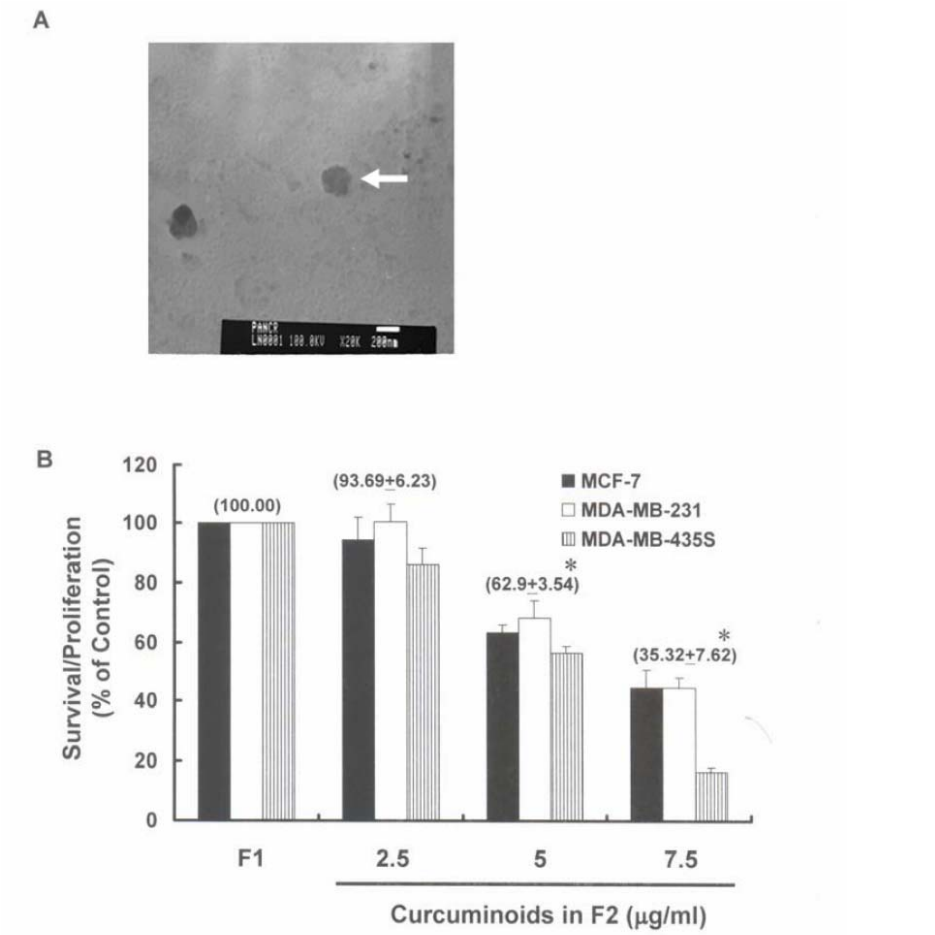

*Fig. 3. (A) LEC were visualized using a transmission electron microscope (model JEM 2000EX, Jeol, Japan); (B) LEC mediates the survival of breast cancer cell lines (MCF-7, MDA-MB-231, and MDA-MB-435S) and thus inhibits proliferation. Each data point represents the mean (±SEM). Statistical analysis used the t-test, with significant differences determined at the level of *P<0.05 versus the F1 group.*

3.2. Liposome formulation could prevent the degradation of curcuminoids by H_2O_2 and light

Curcuminoids could inhibit cancer cell proliferation and the induction of apoptosis in cancer cells via antioxidant activity. H_2O_2 and light destroys the structure of antioxidants and reduces the bioactivity of curcuminoids. The formulation of liposomes significantly reduced the degradation of curcuminoids by H_2O_2 (Fig. 1. A) and exposure to light (Fig. 1. B).

3.3. Influence of preservation temperature and time on particle size and zeta potential in the formulation of liposomes

The influence of preservation temperature (4, 25 and 37℃) and duration in liposome (F2) on the particle size (Fig. 2.A) and zeta potential (Fig. 2. B) was determined over 1, 2, 3, and 4 weeks. The results summarized in Fig. 2A indicate that at 4 and 25 ℃ the particle size was not significantly different from the control (day 0). As shown in Fig. 2B, the zeta potential of F2 decreased with time; however, 4 ℃ was a better preservation temperature for the storage of liposome. Therefore, the samples in this study were preserved at 4 ℃, and used within one month.

3.4. Proliferation of breast cancer cell lines was inhibited by LEC

In this series of studies, a hypothesis was proposed: LEC is capable of mediating the survival of breast cancer cells, and should therefore inhibit proliferation (Table 2). To validate this hypothesis and explore the anti-tumor activity of LEC against breast cancer cells, we initiated an

in vitro study in which each of the three breast cancer cell lines was treated with increasing doses of curcuminoids in F2 (0, 2.5, 5, and 7.5 μg/ml) for 24 h. The proliferation of the LEC-treated cancer cells was then assayed using an MTS test. The results summarized in Fig. 3B indicate that the survival and proliferation of breast cancer cells reduced with an increase in the dose of curcuminoids. The survival and proliferation of breast cancer cells demonstrated a dose-dependent reduction in all three cell lines. Moreover, inhibition of proliferation was found to vary according to the type of breast cancer cells, that is: breast cancer cell line MDA-MB-435S (y = 127.41e-0.2318x; R2 = 0.847) was more sensitive to the anti-proliferative action of LEC than those from the MCF-7 (y = 109.76e-0.1125x; R^2 = 0.9314) and MDA-MB-231 (y = 113.31e-0.1121x; R^2 = 0.8824) cell lines following 24-hr exposure to LEC.

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

Cell lines	Cur (μg/ml)	LEC (μg/ml)	LCC (μg/ml)
MCF-7	14.74±1.27	6.68±0.56 *#	19.21±0.48
MDA-MB-231	9.24±0.79	6.88±0.37 *#	18.09±0.98
MDA-MB-435S	6.90±1.43	5.21±0.17 #	30.37±0.96

LEC: Liposome encapsulated curcuminoids
LCC: Liposome co-treated curcuminoids
Cur:curcuminoids free form

(Mean±SD, N=3)
* Vs. Cur P<0.05
# Vs. LCC P<0.05

3.5. Induction of Apoptosis-Dependent Cell Death by LEC in breast cancer cell lines

To further elucidate the anti-cancer mechanism of LEC in breast cancer cell lines, we performed studies on apoptosis. After treating the cells with various doses of curcuminoids loaded LEC (2.5, 5.0 and 7.5 μg/mL), the percentage of apoptotic cells was assessed using Annexin V-FITC and propidium iodide staining, followed by flow cytometric analysis (Fig. 4A). A dot-plot of Annexin V-FITC fluorescence versus PI fluorescence indicates a significant increase in the percentage of apoptotic cells treated with curcuminoids loaded LEC. A significant increase in the percentage of cells undergoing apoptosis was observed at concentrations of 5.0 and 7.5 μg/mL curcuminoids loaded LEC (Fig. 4 B).

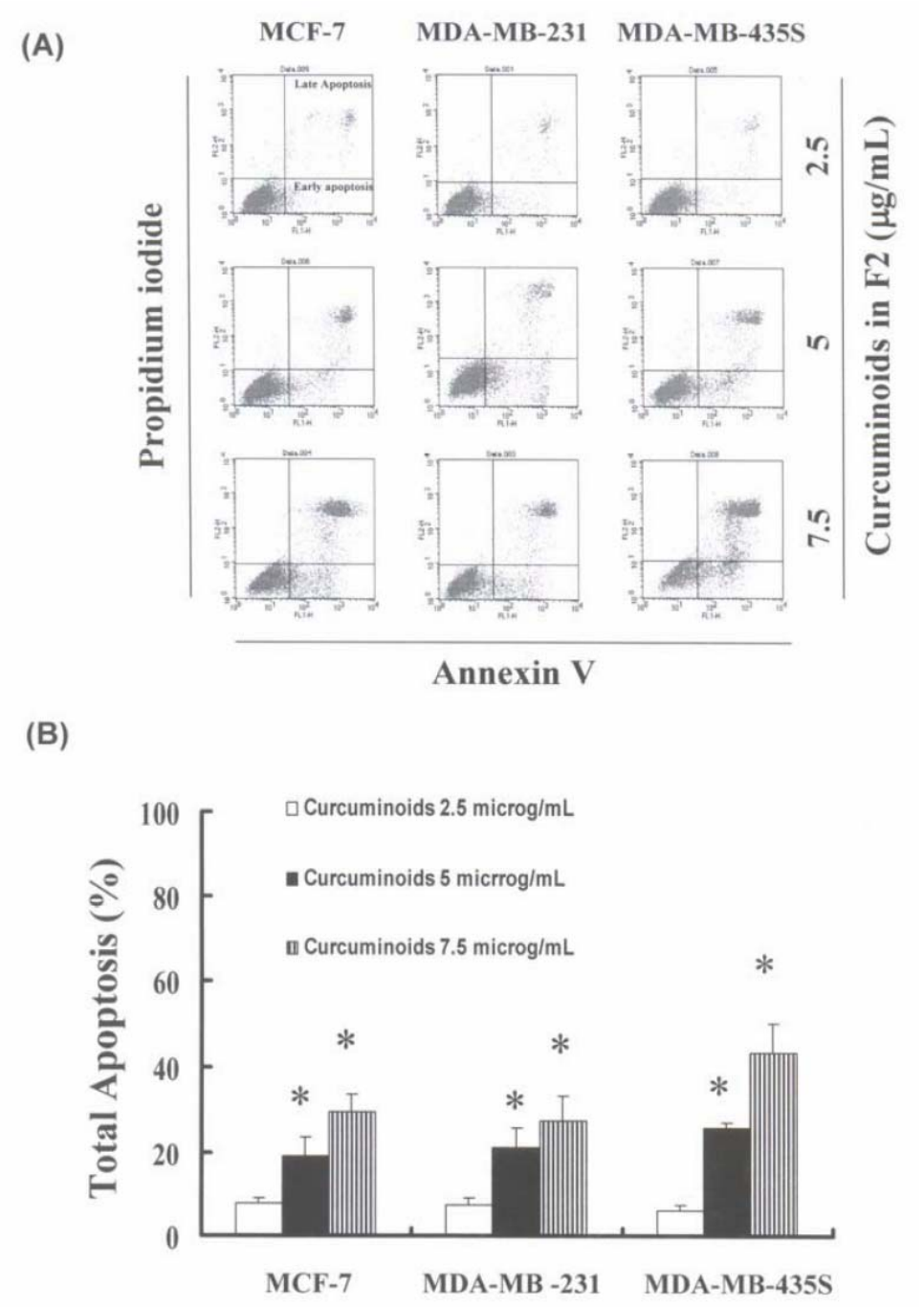

*Fig. 4. LEC induces apoptosis in breast cancer cell lines: (A) cells various different doses curcuminoids (2.5, 5.0, and 7.5 μg/mL) of LEC, the percentage of apoptotic cells was assessed using Annexin V-FITC and propidium iodide staining, followed by flow cytometric analysis; (B) Analysis of total apoptosis (early and late) of breast cancer cell lines after culturing with LEC. All data are reported as the mean (±SEM) of at least three separate experiments. Statistical analysis used the t-test, with significant differences determined at the level of *P<0.05 versus the 2.5 μg/mL LEC group.*

3.6. HPLC method for the assay of cellular curcuminoids released from LEC

To characterize the kinetics involved in the uptake of curcuminoids in LEC by breast cancer cells, we developed an HPLC method to quantitate the level of curcuminoids taken up by the cancer cells following each treatment. Using the HPLC system and conditions outlined in the

Methods section, we identified three well-defined chromatographic peaks representing the major components of curcuminoids: curcumin, the principal constituent, and its demethoxylated derivatives (dihydrocurcumin, or DMC and bisdemethoxycurcumin, or bDMC), [14]. These results imply that the HPLC method in this study is capable of providing quantitative, reproducible assays of curcumin and its demethoxylated derivatives (DMC and bDMC) (data not shown). At concentrations of 5 and 7.5 µg/mL curcuminoids loaded LEC, a significant increase in cellular uptake in human breast cancer cell lines was observed (Table 3). Confocal imaging was used to determine the cellular uptake of LEC. MDA-MB-231 human breast cancer cells were incubated with Rhodamine 123 (red) and LEC was green fluorescein tagged. Figure 5 shows the LEC delivery system transport uptake of tumor cells.

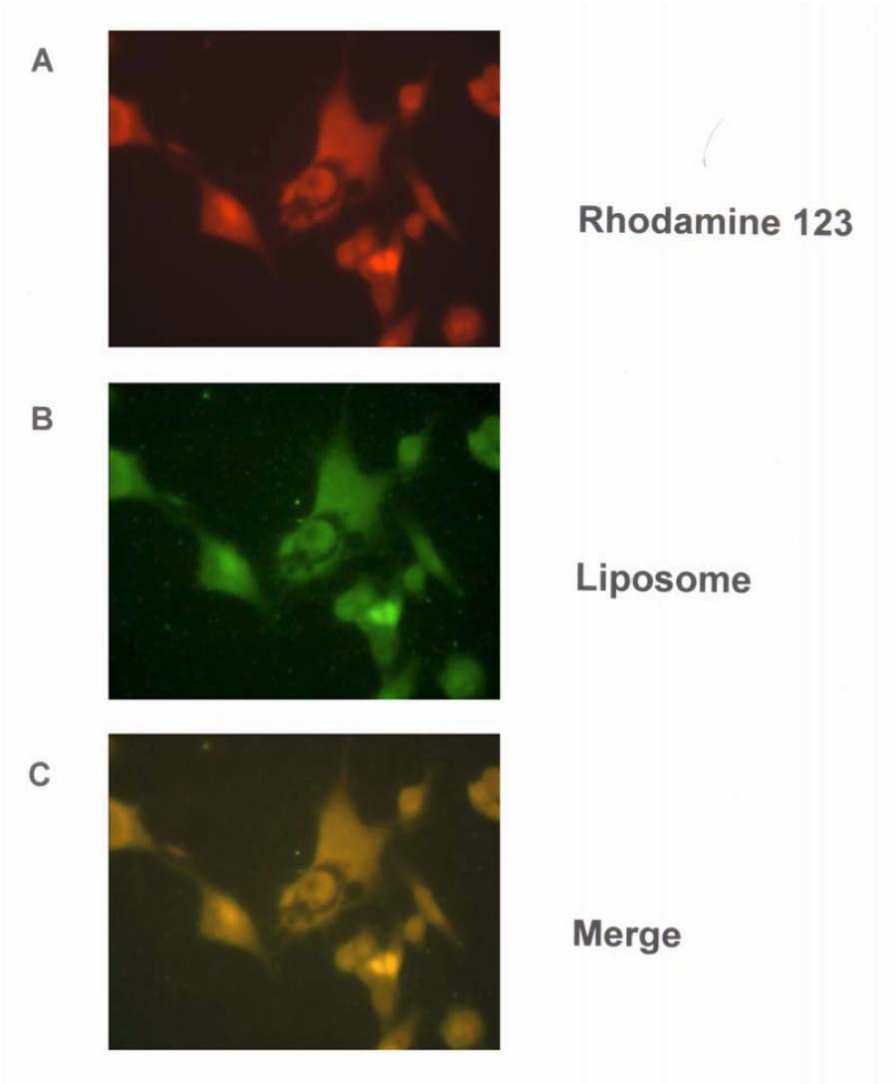

Fig. 5. Confocal imaging of cellular uptake following LEC treatment in MDA-MB-231 human breast cancer cells. Cells were incubated with Rhodamine 123 (red) and LEC was tagged with green fluorescence.

Table 3. Effect of liposomal encapsulation on the cellular uptake of curcuminoids in human breast cancer cell lines.

Cell lines	Cur			LEC			LCC			(μg/ml)
	1.25	2.5	5	1.25	2.5	5	1.25	2.5	5	
MCF-7	ND	12.9±0.1	28.5±0.2	ND	16.8±0.7*	35.2±0.7*#	ND	16.3±0.1	33.3±0.1	
MDA-MB-231	ND	16.0±0.7	34.9±0.1	ND	18.0±0.1*#	42.7±0.2*#	ND	15.4±0.1	32.6±0.1	
MDA-MB-435S	ND	17.9±0.1	36.2±0.1	ND	20.9±0.1*#	42.3±0.2*#	ND	17.8±0.1	39.4±0.4	

LEC: Liposome encapsulated curcuminoids
LCC: Liposome co-treated curcuminoids
Cur:curcuminoids free form

(Mean±SD, N=3)
* Vs. Cur P<0.05
# Vs. LCC P<0.05

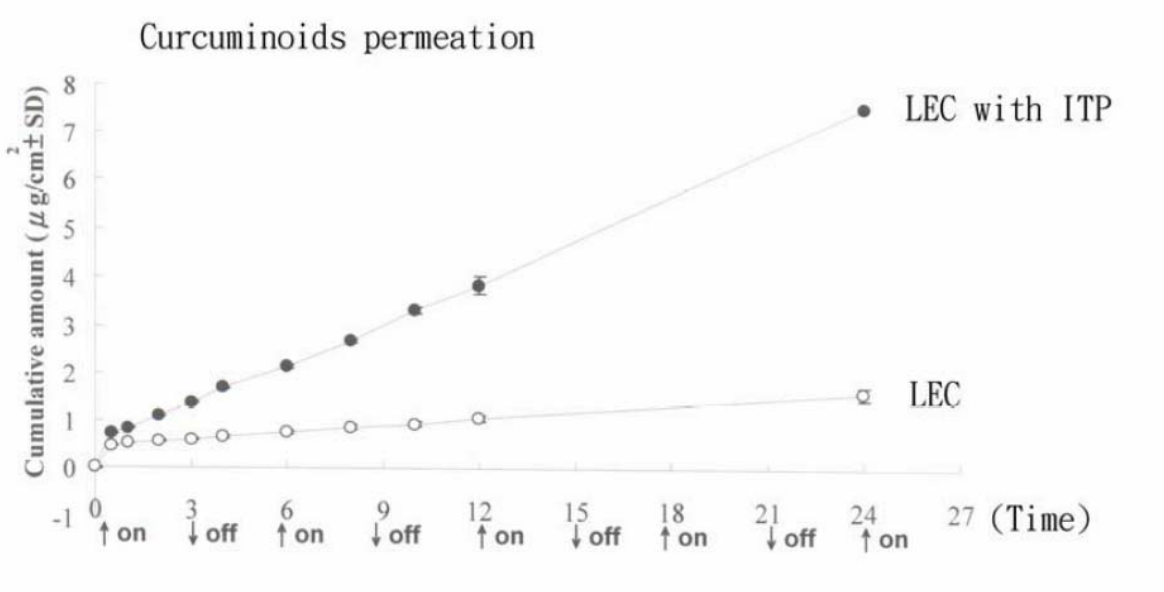

Fig. 6. Permeation profile of curcuminoids on a newborn pig skin. The effect of liposomal encapsulation and ITP current (0.5 mA/cm^2), applied at a pulse pattern (3-hrs on & 3-hrs off), for 24 h. Each data point represents the mean (±SEM) of at least triplicate studies.

3.7. In-vitro skin permeation of ITP

In skin permeation studies on a newborn pig skin, iontophoresis was found to elevate the cumulative amount and flux of LEC (Fig. 6), with a fivefold increase compared to curcuminoids without liposomal encapsulation (7.52 vs. 1.60 μg/cm^2). The ITP showed a significant increase in the cumulative permeation and Flux of LEC (Table 4).

Table 4. The skin permeation kinetics of curcuminoids and LEC

Formulation	ITP	Cumulative permeation in 24 hrs (μg/cm²±SD)	Flux (μg/cm²/hr±SD)
Curcuminoids in solution	+	1.60±0.14	0.067±0.006
	-	1.59±0.27	0.044±0.011
Curcuminoids In liposome	+	7.52±0.18*	0.313±0.008**
	-	0.45±0.36	0.019±0.015

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

4. Discussion

Systemic administration of chemotherapeutic agents results in indiscriminate drug distribution and severe toxicity [18]. Currently, 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. Currently, considerable research is being conducted to design novel nanocarriers capable of detecting cancer in the earliest stages [19], pinpointing its location within the body, and delivering anticancer drugs specifically to malignant cells [20]. Nanocarriers have already proven their ability to deliver therapeutic agents to target cells with the potential to radically alter cancer therapy by increasing the number of effective therapeutic agents [21]. Direct delivery of the drugs to the target tissue allows for reduced doses of toxic substances. Because liposomes can be targeted, the selectivity of drugs toward cancer cells is increased and toxicity to normal tissue is reduced.

Curcuminoids have demonstrated anti-cancer activity and apoptosis in a number of cell types [22]. The results collected in this series of studies with the cell lines of breast adenocarcinoma and breast ductal carcinoma (isolated from breast cancer patients) have provided experimental evidence indicating that liposome encapsulated curcuminoids are capable of irreversibly inducing apoptosis in breast cancer cells.

Surface modification of nanocarriers (liposomes) 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 breast cancer cells, we selected three breast cancer cell lines (MCF-7, MDA-MB-435S and MDA-MB-231). The viability of breast cancer cells was found to decrease with an increase in the loading level of curcuminoids in the liposome. The IC_{50} value of LEC was shown to be lower than that of non-encapsulated curcuminoids (Cur) in breast cancer cell lines (Table 2). These results suggest that liposome enhanced the cellular uptake of curcumin into the breast cancer cells. Liposomal encapsulation also demonstrated a sustained anti-proliferation effect. In addition, this study developed an HPLC technique for quantitateing the cellular uptake of curcuminoids (Table 3). Results indicate that the cellular uptake of curcuminoids by breast cancer cells is dependent on the concentration of curcuminoids in LEC. The cellular uptake of curcuminoids in the liposomal formulation was higher than that of free-form curcuminoids.

5. Conclusions

The use of liposomal curcuminoids (LEC) as a transdermal delivery system with iontophoresis shows considerable potential for a wide range of breast cancer research purposes and clinical applications.

Acknowledgment

The authors would like to express their appreciation for the funding support provided by the National Science Council (Taiwan) (grant NSC94-2745-B-037-011-URD) and E-Da Hospital, E-Da Hospital/ I-Shou University (grant EDAHP100018). The authors are also grateful to the technical assistance provided by Prof. Yie W. Chien of InnovaTherapeutics Research Center in Kaohsiung Medical University.

References

[1] S. Bengmark, M.D. Mesa, A. Gil, Nutr Hosp. **24**, 273 (2009).
[2] W.Y. Huang, Y.Z. Cai, Y. Zhang, Nutr Cancer. **62**, 1 (2010).
[3] T. Osawa, Y. Kato, Ann N Y Acad Sci. **1043**, 440 (2005).
[4] M.T. Huang, H.L. Newmark, K. Frenkel, J Cell Biochem Suppl. **27**, 26. (1997).
[5] M.A. Perazella, G.W. Moeckel, Semin Nephrol. **30**, 570 (2010).
[6] R, Mallipeddi, L,C. Rohan, Int J Nanomedicine. **5**, 533 (2010).
[7] B.D. Kurmi, J. Kayat, V. Gajbhiye, R.K. Tekade, N.K. Jain, Expert Opin Drug Deliv. **7**, 781 (2010).
[8] W. Jiang, R. Lionberger, L.X. Yu, Bioanalysis. **3**, 333 (2011).
[9] M.R. Mozafari, A. Pardakhty, S. Azarmi, J.A. Jazayeri, A. Nokhodchi, A. Omri, J Liposome Res. **19**, 310 (2009).
[10] H. Zhang, G. Wang, H. Yang, Expert Opin Drug Deliv. **8**, 171 (2011).
[11] R. Csuk, A. Barthel, R. Sczepek, B. Siewert, S. Schwarz, Arch Pharm. **344**, 37 (2011).
[12] S. Rathod, S.G. Deshpande, Indian J Pharm Sci. **72**, 155 (2010).
[13] L.N. Ramana, S. Sethuraman, U. Ranga, U.M. Krishnan. J Biomed Sci. **17**, 57 (2010).
[14] Y.C. Hsu, H.C. Weng, S. Lin, Y.W. Chien J Agric Food Chem. **55**, 8213 (2007).
[15] T.Y. Huang, T.H. Tsai, C.W. Hsu, Y.C. Hsu, J Agric Food Chem. **58**, 10639 (2010).
[16] L. Wan, X. Zhang, S. Pooyan, M.S. Palombo, M.J. Leibowitz, S. Stein, P.J. Sinko, Bioconjug Chem. **19**, 28 (2008).
[17] M.I. Papisov, A. Yurkovetskiy, S. Syed, N. Koshkina, M. Yin, A. Hiller, A.J. Fischman, Mol Pharm. **2**, 47 (2005).
[18] E. Allard, C. Passirani, J.P. Benoit, Biomaterials. **30**, 2302 (2009).
[19] S. Ganta, H. Devalapally, A. Shahiwala, M. Amiji, J Control Release. **126**, 187 (2008).
[20] T. Minko, R.I. Pakunlu, Y. Wang, J.J. Khandare, M. Saad, Anticancer Agents Med Chem. **6**, 537 (2006).
[21] C. Mohanty, M. Das, J.R. Kanwar, S.K. Sahoo, Curr Drug Deliv. **8**, 45 (2011).
[22] M.J. Chen, Y.Y. Chu, P.H. Lai, Y.M. Cheng, Y.C. Hsu, Dig J Nanomater Bios. **6**, 1187 (2011).