



Cytotoxicity evaluation of curcumin-loaded affibody-decorated liposomes against breast cancerous cell lines

Mahshid Moballegh-Nasery, Ali Mandegary, Toubia Eslaminejad, Majid Zeinali, Abbas Pardakhti, Behzad Behnam & Mohadeseh Mohammadi

To cite this article: Mahshid Moballegh-Nasery, Ali Mandegary, Toubia Eslaminejad, Majid Zeinali, Abbas Pardakhti, Behzad Behnam & Mohadeseh Mohammadi (2020): Cytotoxicity evaluation of curcumin-loaded affibody-decorated liposomes against breast cancerous cell lines, Journal of Liposome Research, DOI: [10.1080/08982104.2020.1755981](https://doi.org/10.1080/08982104.2020.1755981)

To link to this article: <https://doi.org/10.1080/08982104.2020.1755981>



Accepted author version posted online: 15 Apr 2020.
Published online: 03 Jul 2020.



Submit your article to this journal [↗](#)



Article views: 23



View related articles [↗](#)



View Crossmark data [↗](#)

ARTICLE



Cytotoxicity evaluation of curcumin-loaded affibody-decorated liposomes against breast cancerous cell lines

Mahshid Moballegh-Nasery^{a,b} , Ali Mandegary^{b,c} , Touba Eslaminejad^b , Majid Zeinali^d ,
Abbas Pardakhti^b , Behzad Behnam^{b,e}  and Mohadeseh Mohammadi^f 

^aStudent Research Committee, Kerman University of Medical Sciences, Kerman, Iran; ^bPharmaceutics Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran; ^cDepartment of Toxicology & Pharmacology, School of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran; ^dBiotechnology Research Center, Research Institute of Petroleum Industry (RIPI), Tehran, Iran; ^eDepartment of Pharmaceutical Biotechnology, School of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran; ^fDepartment of Animal Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran

ABSTRACT

Curcumin is known as an effective anticancer herbal medicine but unfortunately, its bioavailability is poor which necessitate efforts for developing more efficient and specific delivery systems. Human epidermal growth factor receptor 2 (HER 2) due to its overexpression in various types of cancers, is demonstrated to be a good candidate as a target for anticancer therapy. In this study, cytotoxicity of curcumin encapsulated in ZHER2:342 Affibody-decorated liposome was investigated against SKBR3 and MCF-7 cancerous cell lines. Curcumin-containing liposomes were prepared from soybeans lecithin and cholesterol by thin-film hydration method. Affibody ZHER2:342 molecules *via* C-terminal cysteine residue were conjugated covalently to the prepared liposomes. Particle size analysis was performed using atomic force microscopy (AFM) and dynamic light scattering (DLS). Curcumin loading was measured using UV-Vis spectrophotometry and cytotoxic activity of curcumin formulations against cancerous cell lines was investigated by MTT assay. Induction of apoptosis was investigated using flow cytometry through Annexin V staining. Particle analysis showed the formation of spherical liposomes with a mean diameter of about 150 nm. Cytotoxic activity of curcumin was improved by its encapsulation in both liposomes and affibody-decorated liposomes. The Annexin V staining indicated the induction of apoptosis by affibody-decorated liposomes in both MCF-7 and SKBR3 cells. Decoration of curcumin-loaded liposomes with affibody ZHER2:342 may improve curcumin apoptotic function independently of HER2 expression level.

ARTICLE HISTORY

Received 18 September 2019
Revised 5 April 2020
Accepted 12 April 2020

KEYWORDS

Curcumin; HER2; breast cancer; affisome; targeted therapy

1. Introduction

When normal cells lose their cellular homeostasis and proliferation of genes involved in cell cycle, invasion, survival, metastasis, and angiogenesis is unusually activated, cancer occurs (Shanmugam *et al.* 2015). One of the most commonly diagnosed cancers in the world is breast cancer with more than one million new cases each year. Tumour removal followed by chemotherapy or radiation therapy is the most common approach in treatment of patients with breast cancer (Dhule *et al.* 2012). Nonspecificity, toxic effects on normal cells and drug/radio resistance are the main challenges of these approaches in cancer treatment (Zokaei *et al.* 2018). The main challenge in cancer treatment is providing a therapeutic window to kill cancer cells without damage to normal cells (Zahmatkeshan *et al.* 2016). Specific delivery of cytotoxic payloads to the solid tumours is the best choice but finding specific targets on cancerous cells and designing targetting moiety on drug cargos are the main problems (Zahmatkeshan *et al.* 2016, Sochaj-Gregorczyk *et al.* 2017).

Biological agents and natural herbal extracts for a long time have been used for prevention of diseases and infection conditions (Eslaminejad *et al.* 2012a, 2012b, Ranjan *et al.* 2013, Ohadi *et al.* 2017). The rhizomes of the perennial herb *Curcuma longa* is used as a spice in traditional medicine in Asia (Allijn *et al.* 2016). Curcumin as the yellow pigment of turmeric has shown a wide range of bioactivities specially anti-tumour effects in both *in vitro* and *in vivo* experiments (Barui *et al.* 2014). It is shown that curcumin may interact with transcription factors, tumour necrosis factor alpha (TNF- α), tumour suppressor genes and also enzymatic systems involved in carcinomas including cyclooxygenases and lipoxygenases (Ibrahim *et al.* 2018). But, low aqueous solubility, poor bioavailability and rapid metabolism in the human body are the main problems of curcumin administration in the cancer therapy (Chen *et al.* 2012). To overcome these problems, curcumin was encapsulated in liposome (Alam *et al.* 2012). Liposomes due to their unique characteristics such as small size, hydrophobic/hydrophilic nature, biodegradability and low toxicity have been widely used as

drug delivery system (Chen *et al.* 2012). However, the most disadvantage of the liposomal delivery systems is their lack of selectivity (Catania *et al.* 2013).

Monoclonal antibodies (mAbs) because of strong affinity to their ligands can be employed as tumour-specific targeting moieties for specific delivery of drug cargoes to tumour cells (Löfblom *et al.* 2010). The large size of mAbs (molecular weight of about 150 kDa) and its consequences including rapid blood clearance, poor tissue penetration, and high liver uptake impose certain limitations (Serwotka-Suszczak *et al.* 2017). An option to overcome these limitations is designing new small high-affinity peptide moieties as targeting agents (Altai *et al.* 2014).

Affibody as a member of antibody mimics family is candidate of choice for this purpose. These engineered peptides, which are derived from the Z domain of staphylococcal protein A, have small size (molecular weight of about 6.5 kDa) and short plasma retention time permitting their rapid tissue distribution (Trousil *et al.* 2014). Affibody molecules are stable and water-soluble and can be readily expressed in bacterial expression systems (Lee *et al.* 2008). Affibodies selected against the human epidermal growth factor receptor 2 (HER2) are among the most studied affibody molecules to date (Sochaj-Gregorczyk *et al.* 2017).

HER2 is a transmembrane protein and belongs to the receptor tyrosine kinase superfamily. Overexpression of HER2 has been reported in various types of cancers and is associated with poor prognosis in breast, ovary, colorectal, and urothelial carcinomas. Due to its overexpression in some types of cancers, HER2 can be considered as a good candidate for directing affibody conjugated-drugs towards cancer cells (Ahlgren *et al.* 2009). In the current study, curcumin was encapsulated either in bare liposomes or affibody ZHER2:342-decorated liposomes (affisomes). The cytotoxic activity of the prepared affisomes compared to the free and liposomal curcumin against two breast cancer cell lines, MCF-7 and SKBR3 was investigated through MTT assay and Annexin V staining.

2. Materials and method

2.1. Materials

3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), and Triton X-100 were purchased from Sigma Chemical Co. (St Louis, MO). Curcumin was purchased from Merck (Darmstadt, Germany). Soybean lecithin and cholesterol were obtained from Applichem (Darmstadt, Germany). Maleimide-terminated DSPE-PEG₂₀₀₀ (1, 2-distearoyl-Sn-glycero-3-phosphoethanolamine-N-[maleimide (polyethylene glycol)-2000]) was purchased from Avanti Polar Lipids (Alabaster, AL). Dulbecco's modified Eagle's high glucose medium (DMEM), RPMI 1640, and foetal bovine serum (FBS) were purchased from Biosera (France). Penicillin-streptomycin and trypsin-EDTA were purchased from GIBCO. Annexin V-FITC PI Apoptosis Assay kit was purchased from IQ Products (Rozenburglaan, Netherlands) and all other solvents and reagents were of analytical or HPLC grade and were obtained from Merck.

2.2. Preparation of curcumin-loaded liposomes

Soybean lecithin and cholesterol at a molar ratio of 3:2 were used for preparation of liposomes by lipid film hydration method. Lipid components and curcumin were dissolved in chloroform: methanol (9:1 v/v) solvent mixture. The curcumin: lipid molar ratio was 1:5. The organic solvent was evaporated under a stream of N₂ gas to obtain a dry lipid film. Any residual organic solvent was removed under vacuum desiccation for 2 h. The lipid film was hydrated with phosphate buffered saline (PBS, pH 7.4) under vigorous stirring at 55 °C for 30 min to obtain a final lipid concentration of 10 µmol ml⁻¹. The milky suspension was sonicated in a bath sonicator (Elmasonic P, Germany) for 10 min at 55 °C.

2.3. Preparation of curcumin-loaded affisome

De novo gene synthesis, expression and purification of ZHER2:342 affibody was conducted according to our previous publication (Ghanemi *et al.* 2018). For covalent conjugation of Affibody ZHER2 to phospholipid components of the liposome and preparation of affisome, maleimide-terminated DSPE-PEG₂₀₀₀ was included in the lipid mixture of liposomal formulation at 2 mol % of the total lipids. For affisome construction, the prepared liposomes were incubated with affibody ZHER2:342 in the reaction buffer (50 mM sodium phosphate, 150 mM NaCl and 1 mM EDTA, pH 7.5) at room temperature. Unbound affibody molecules and free curcumin were separated from liposomes by gel filtration chromatography using Sephacryl S-100HR.

2.4. Characterization of the liposomes and affisomes

The morphology and size of the prepared lipid vesicles were determined by atomic force microscopy (AFM, Nano wizard[®] nano-optics AFM system, JPK, Berlin, Germany). Dynamic light scattering (DLS) was performed using Brookhaven 90 plus particle size analyser (Brookhaven Instruments Corporation) to measure the hydrodynamic size, charge and also polydispersity of the constructed lipid vesicles. Curcumin content of the liposomal formulations was determined after disruption of the lipid vesicles by Triton X-100 solution (1% w/v). Briefly, liposomal curcumin was mixed with an equal volume of Triton X-100 solution and after dilution (20 folds), optical density (OD) was measured by using Specord 210 plus UV-Vis spectrophotometer (Analytik Jena, Germany) at the wavelength of 425 nm. Curcumin concentration was determined based on the standard curve. Presence of ZHER2:342 affibody on the surface of affisomes was confirmed by the appearance of protein 280 nm characteristic absorption band in the UV-Vis spectrum of the affibody-decorated liposomes. The protein content of the affisome preparation was determined using Bradford reagent against BSA as a standard.

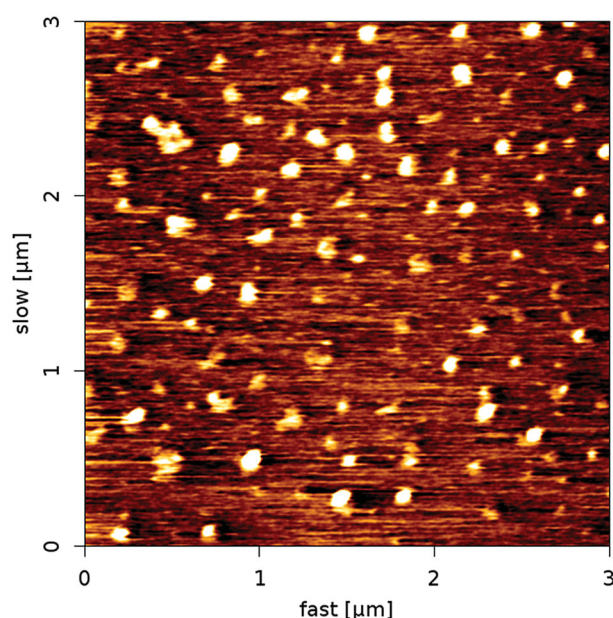


Figure 1. Atomic force microscopy (AFM) analysis of curcumin-loaded liposomes. Mean diameter of liposomal vesicles were estimated 100 nm.

2.5. Cell culture and cell viability assay

Human breast cancer SKBR3 and MCF-7 cell lines were cultured in 2% FBS-containing RPMI or DMEM medium supplemented with penicillin/streptomycin ($100 \mu\text{g ml}^{-1}$) and incubated in a humidified atmosphere of 5% CO_2 at 37°C . Cells were seeded at a density of 7×10^3 cells well^{-1} in 96-well plates and incubated for 24 h. Then, the medium was removed and the cultured cells were treated by different forms of curcumin (free or liposomal) at various concentrations of 0.312, 0.625, 1.25, 2.5, 5 and $7.5 \mu\text{g ml}^{-1}$ for a duration time of 24 or 48 h. Cell survival was determined by adding $20 \mu\text{l}$ of MTT solution (5 mg ml^{-1}) to each well followed by incubation for additional 3 h at 37°C . The absorbance of the isopropanol-solubilized blue-coloured product (formazan) was measured at 570 nm using a microplate reader (Biotech) and the percent viability of the cell was calculated compared to the control untreated corresponding cells.

2.6. Apoptosis assay

Cells were seeded at a density of 10^5 cell well^{-1} in six-well plates and then treated with different forms of curcumin (free or liposomal). After 48 h of incubation, the treated cells were washed with PBS, trypsinized, stained using Annexin V-FITC PI kit according to the supplier's instruction and finally analysed by flow cytometry (Partech flow cytometry, Germany). Due to intrinsic fluorescence of curcumin and possibility of its overlapping with emission spectrum of FITC, curcumin and curcumin-loaded liposomes without FITC-annexin V were used as control FACS experiments to correct for curcumin background in FACS analysis.

2.7. Data analysis and statistical method

All experiments were performed in triplicate and in three independent assays. Data were expressed as mean $\pm$ standard deviation (SD). The statistical analysis of the data was performed with student t-test using SPSS 18.0 software and values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Characteristics of curcumin-loaded liposomes and affisomes

The mean diameters of curcumin-loaded liposomes and affisomes as determined using DLS were 145 nm (polydispersity of 0.148) and 157 nm (polydispersity of 0.199), respectively. Based on the micrographs prepared by AFM, liposomes and affisomes had a smooth surface with spherical shape. Also, according to the results of the AFM analysis, diameter of the liposomes and affisomes were in the range of 90–110 nm (Figure 1).

Encapsulation of curcumin in the constructed lipid vesicles was confirmed by UV-Vis spectroscopy of Triton-disrupted vesicles at the absorption peak of curcumin (424 nm). Based on a standard curve, the mean concentration of curcumin in the prepared liposomes and affisomes was about $180 \mu\text{g ml}^{-1}$. Conjugation of ZHER2:342 affibody on the surface of lipid vesicles was confirmed by the appearance of protein characteristic band of 280 nm in the UV-Vis spectrum of the affisomes. The protein content of the curcumin-loaded affisomes as determined by Bradford reagent was 0.25 mg ml^{-1} . Based on our calculations, about 10% of the reactive lipid molecules in the affisome preparation were in conjugation with affibody molecules.

3.2. Evaluation of the cytotoxic activity of curcumin-loaded liposomes and affisomes

The cytotoxic activity of curcumin, free or encapsulated in the lipid vesicles, against two breast cancer cell lines SKBR3 and MCF-7 was investigated using MTT assay (Figure 2). Effect of curcumin on viability of breast cancer cells was time and dose-dependent. The $\text{LC}_{50}/24 \text{ h}$ and $\text{LC}_{50}/48 \text{ h}$ values for free curcumin in both MCF-7 (Figure 2(a)) and SKBR3 (Figure 2(b)) cells were more than $7.5 \mu\text{g ml}^{-1}$. Formulation of curcumin in the liposomal form decreased its LC_{50} value in both MCF-7 and SKBR3 cell lines. The $\text{LC}_{50}/24 \text{ h}$ and $\text{LC}_{50}/48 \text{ h}$ values for liposomal curcumin against MCF-7 cell line were 7.5 and $5.0 \mu\text{g ml}^{-1}$, respectively (Figure 2(c)). For liposomal curcumin against SKBR3 cell line, $\text{LC}_{50}/24 \text{ h}$ value was not determined in the examined concentration range and the $\text{LC}_{50}/48 \text{ h}$ value was between 2.5 – $5.0 \mu\text{g ml}^{-1}$ (Figure 2(d)). Conjugation of affibody ZHER2:342 on the surface of lipid vesicles to prepare curcumin-loaded affisomes have no remarkable effect on the potential of curcumin in reducing viability of SKBR3 cells (Figure 2(f)). Both the liposomal (Figure 2(d)) and affisomal (Figure 2(f)) forms of curcumin reduced the viability of SKBR3 cells to 40% after 48 h in a concentration of $5.0 \mu\text{g ml}^{-1}$. The affisomal form of curcumin

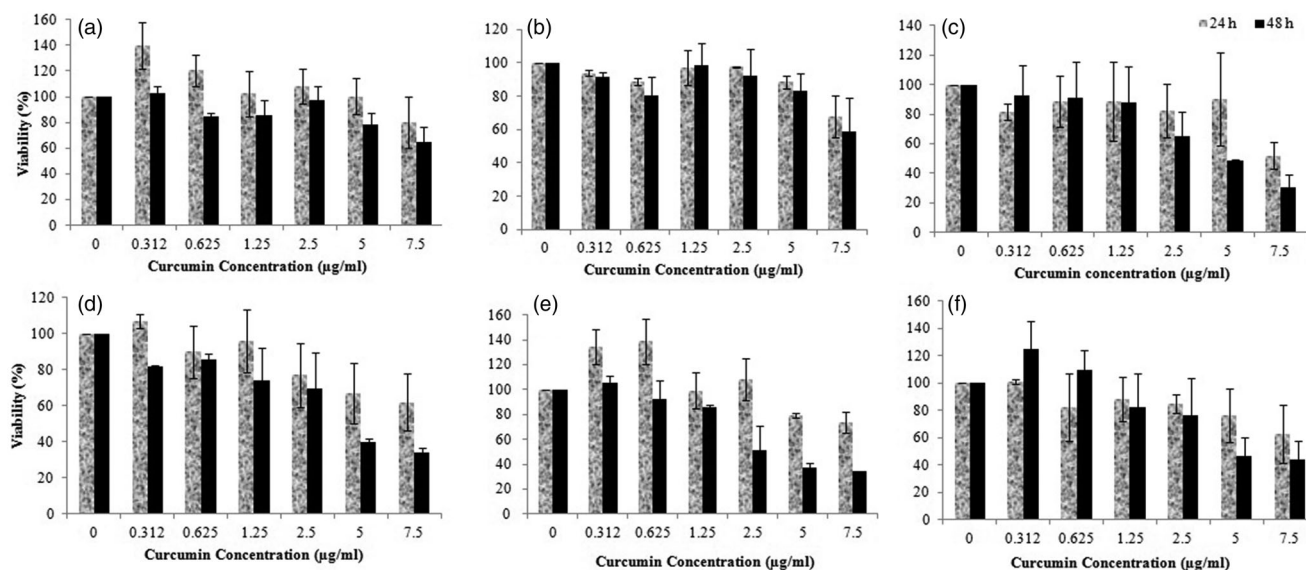


Figure 2. Cytotoxicity of free, liposomal and affisomal forms of curcumin. Cell lines SKBR3 and MCF-7 were treated with different concentrations of curcumin (0–7.5 µg ml⁻¹) in three different forms for 24/48 h and then cell viability was evaluated by MTT assay. (a) MCF-7 cells treated with free curcumin, (b) SKBR3 cells treated with free curcumin, (c) MCF-7 cells treated with liposomal curcumin, (d) SKBR3 cells treated with liposomal curcumin, (e) MCF-7 cells treated with affisomal curcumin, (f) SKBR3 cells treated with affisomal curcumin. Light and dark bars representative of 24 and 48 hours, respectively.

(Figure 2(e)) compared to the liposomal form (Figure 2(c)) was more potent in decreasing viability of MCF-7 cells. The LC₅₀/48h value for the affisomal curcumin against MCF-7 cell line was 2.5 µg ml⁻¹ (Figure 2(e)).

3.3. Apoptosis analysis

The ability of free curcumin and the curcumin-loaded liposome/affisome to induce apoptosis in MCF-7 and SKBR3 cells was investigated by performing Annexin V/PI assay. In this assay, viable cells are both Annexin V and PI negative (V⁻PI⁻) while the cells in the early stages of apoptosis are Annexin V positive and PI negative (V⁺PI⁻). The cells which are both Annexin V and PI positive (V⁺PI⁺) are in the late apoptosis or death stage. In the control untreated samples, the majority of either MCF-7 or SKBR3 cells were viable after 48 h of incubation. Treatment of cells with free curcumin (2 µg ml⁻¹ for 48 h) resulted in a mild increase of apoptotic cell population (about 7.0% in MCF-7 cells and 20% in SKBR3 cells) (Figure 3). When the cells were treated with liposomal curcumin, the percentages of apoptotic cells were about 22% (MCF-7 cells) and 25% (SKBR3 cells). In the affisomal curcumin-treated MCF-7 and SKBR3 cells, percentage of apoptotic cell populations were about 37%. This means that, the apoptotic cells were significantly increased in SKBR3 and MCF7 cells treated with curcumin-loaded affisomes compared to the other groups. No significant difference between SKBR3 and MCF-7 cell lines treated with the curcumin-loaded affisomes was observed with respect to apoptosis induction.

4. Discussion

Cancer as the second leading cause of death in the world is responsible for 9.6 million deaths in 2018 according to WHO report. After lung cancer, breast cancer with 2.09 million new

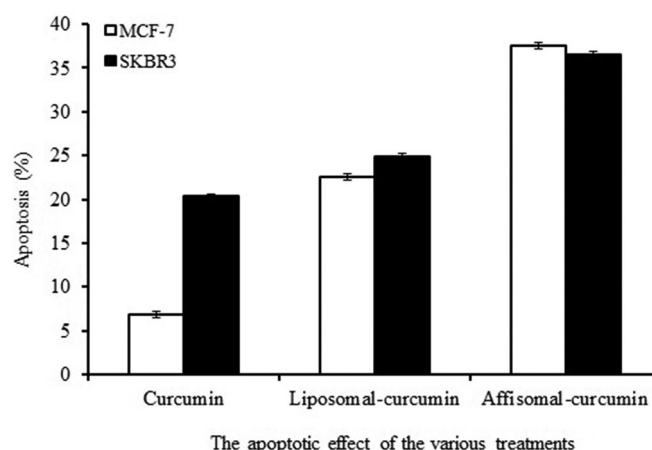


Figure 3. Induction of apoptosis by free, liposomal and affisomal curcumin. Cells were seeded at a density of 10⁵ cell well⁻¹ in six-well plates and then treated with free, liposomal or affisomal curcumin. After 48 h of incubation, the treated cells were stained using Annexin V-FITC PI kit and analyzed by flow cytometry. Light and dark bars are representative of MCF-7 and SKBR3 cell lines, respectively.

cases and 627 000 deaths in year 2018 is the most prevalent cancer among females worldwide. Several studies in the last 50 years have shown the chemopreventive properties of curcumin. Based on various *in vitro* and *in vivo* studies, curcumin may be regarded as a therapeutic agent in a wide range of inflammatory disorders and cancers (Salehiabar *et al.* 2018). It is shown that clonogenic growth of MCF-7 and SKBR3 cells is completely inhibited by curcumin in a concentration range of 25–50 µM (Filippi *et al.* 2019). However, due to water insolubility and poor systemic bioavailability, curcumin activity is limited. In recent years, curcumin in different formulation and forms such as nanoparticles, Nano gels, dendrimers, liposomes or micelles has been prepared and studied to overcome poor absorption limitations (Shanmugam *et al.* 2015). Among different formulations,

liposomes have been widely used in drug delivery approaches to cancer cells (Feng *et al.* 2017). Dose-dependent inhibitory effect of nanoliposomal curcumin on breast cancer MCF-7 cell line has been shown (Santosh *et al.* 2012, Hasan *et al.* 2014). The aim of the present study was to enhance cytotoxicity of curcumin *in vitro* against breast cancer cells through its encapsulation into liposomes and also to evaluate selectivity of the affibody ZHER2:342-decorated liposomes to target HER2-overexpressing breast cancer cells such as SKBR3 cells. Measurements were done on SKBR3 and MCF-7 cell lines after treatment with different concentrations of curcumin ($0\text{--}7.5\text{ }\mu\text{g ml}^{-1}$) either free or encapsulated in liposome/affisome. The cell lines were chosen based on their different levels of HER2 expression.

First, the effect of different formulations of curcumin on MCF-7 and SKBR3 cell proliferation was studied using MTT method. As seen in Figure 2, free curcumin showed a similar anti-proliferative effect against both cell lines in a concentration dependent manner. Free curcumin up to $2.5\text{ }\mu\text{g ml}^{-1}$ was non-toxic against both cell lines after 48 h and at higher concentrations some degrees of toxicity independent of the HER2 expression level were seen. At the highest examined concentration ($7.5\text{ }\mu\text{g ml}^{-1}$), curcumin decreased viability of both MCF-7 and SKBR3 cells to about 60% after 48 h. The reported LC_{50} value for free curcumin against MCF-7 cell line is about $20.0\text{ }\mu\text{g ml}^{-1}$ (Santosh *et al.* 2012). Low bioavailability and rapid systemic elimination have forced scientists to search for curcumin formulations with greater growth inhibitory effects. Between different formulations, liposomal curcumin has attracted more attention. In the current study, we prepared curcumin-loaded liposomes with a mean diameter of 145 nm. The size of liposome is an important factor that affects its targeting efficiency and systemic distribution. It is shown that high levels of small PEG liposomes (with diameter less than 200 nm) are observed in the blood while larger liposomes ($\geq 200\text{ nm}$) are mainly accumulated in the spleen (Klibanov *et al.* 1991). Therefore, it can be expected that our prepared curcumin-loaded liposomes may be suitable for targeting cancer cells at *in vivo* conditions.

Anti-proliferative effect of liposomal curcumin against MCF-7 cells was seen after 48 h at a curcumin concentration of $2.5\text{ }\mu\text{g ml}^{-1}$ (Figure 2(c)). In SKBR3 cells, cytotoxic effect of liposomal curcumin was recorded at a curcumin concentration of $1.25\text{ }\mu\text{g ml}^{-1}$ after 48 h (Figure 2(d)). The $\text{LC}_{50}/48\text{ h}$ value for liposomal curcumin against MCF-7 cell line was $5.0\text{ }\mu\text{g ml}^{-1}$ while in the case of SKBR3 cells was less than $5.0\text{ }\mu\text{g ml}^{-1}$. In a previous work reported by Santosh *et al.* (2012), the $\text{LC}_{50}/48\text{ h}$ values of conventional liposomal curcumin and curcumin-loaded- γ -cyclodextrin liposome against MCF-7 cells were 11.5 and $10.2\text{ }\mu\text{g ml}^{-1}$, respectively. Our obtained results in accordance with the previous published reports clearly showed that the liposomal curcumin compared to the free curcumin has greater growth inhibitory effect on the both examined breast cancer cell lines.

One of the main problems in cancer treatment which is often led to radioresistance or chemoresistance is apoptosis resistance in most cancers. Additionally, chemotherapeutic failures can be result in activated immune response following

cell death (Santosh *et al.* 2012) which may be reduced by induction of apoptosis. An approach for improvement of cancer therapy and provide new therapeutic opportunities for patients suffering from malignancies is modulation of apoptosis pathways by natural products including betulinic acid, vitamin E analogues, resveratrol, curcumin and *etc.* (Fulda, 2010). Although cytotoxicity of free curcumin against both MCF-7 and SKBR3 cell lines were comparable (Figure 2) but apoptotic effects of free curcumin against SKBR3 cells was three times stronger than those observed in MCF-7 cells (Figure 3). Encapsulation into liposome improved the apoptotic function of curcumin against MCF-7 cells (3.3-folds) (Figure 3), while no remarkable difference was seen between free and liposomal curcumin in induction of apoptosis in SKBR3 cells.

It is shown that incidence of metastasis is higher in patients with HER2-overexpressing breast tumours compared with patients bearing tumours with normal HER2 expression level (Yan *et al.* 2015). In an attempt to increase doxorubicin delivery to SKBR3 cells, it is shown that $\text{LC}_{50}/3\text{ h}$ value of doxorubicin-loaded affibody ZHER2:477-grafted liposome was lower than conventional liposome (Akhtari *et al.* 2016). In the present study, grafting affibody ZHER2:342 on the surface of curcumin-loaded liposome had no remarkable effect on the LC_{50} values of liposomal curcumin against either MCF-7 or SKBR3 breast cancer cells (Figure 2). But, on the other hand, affibody-decorated curcumin-loaded liposomes compared to conventional liposomes showed an increased apoptotic function against both cell lines. In both cell lines, the percentage of apoptotic cells was reached to about 37% upon treatment with curcumin-loaded affisomes (Figure 3). No significant difference was seen between MCF-7 and SKBR3 cells in response to curcumin-loaded affisomes. In another word, affisomal form of curcumin showed comparable apoptotic effects against both cell lines independently of the HER2 expression levels in the treated cells.

5. Conclusions

Free curcumin is cytotoxic but not apoptotic. The mode of cell death in response to this natural compound may be necrosis which is undesirable in cancer therapy due to induction of immune responses. Formulation of curcumin in liposomal form is a promising approach in delivery of this compound to cancer cells and also to modulate the response of affected cells. Affibody attachment on the surface of curcumin-loaded liposomes led to improvement of its apoptotic function against breast cancer cells independently of the levels of HER2 expression while the cytotoxic effect remained unchanged. This property of affisomal curcumin could be applicable for modulation of apoptosis signalling pathways in cancer therapy.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The present work was part of a MSc. thesis supported by the Kerman University of Medical Sciences (KMU) (grant number: 96001012).

ORCID

Mahshid Moballegheh-Nasery  <http://orcid.org/0000-0001-7662-9161>

Ali Mandegary  <http://orcid.org/0000-0002-1065-9349>

Touba Eslaminejad  <http://orcid.org/0000-0001-5920-1542>

Majid Zeinali  <http://orcid.org/0000-0003-0155-960X>

Abbas Pardakhti  <http://orcid.org/0000-0002-1848-5961>

Behzad Behnam  <http://orcid.org/0000-0002-7338-1911>

Mohadeseh Mohammadi  <http://orcid.org/0000-0003-1062-8849>

References

- Ahlgren, S., et al., 2009. Targeting of HER2-expressing tumors with a site-specifically 99mTc-labeled recombinant affibody molecule, ZHER2: 2395, with C-terminally engineered cysteine. *Journal of nuclear medicine: official publication, society of nuclear medicine*, 50 (5), 781–789
- Akhtari, J., et al., 2016. Targeting, bio distributive and tumor growth inhibiting characterization of anti-HER2 affibody coupling to liposomal doxorubicin using BALB/c mice bearing TUBO tumors. *International journal of pharmaceuticals*, 505 (1–2), 89–95
- Alam, S., Panda, J.J., and Chauhan, V.S., 2012. Novel dipeptide nanoparticles for effective curcumin delivery. *International journal of nanomedicine*, 7, 4207–4222
- Allijn, I.E., Schiffelers, R.M., and Storm, G., 2016. Comparison of pharmaceutical nanoformulations for curcumin: Enhancement of aqueous solubility and carrier retention. *International journal of pharmaceuticals*, 506 (1–2), 407–413
- Altai, M., et al., 2014. 188Re-ZHER2: V2, a promising affibody-based targeting agent against HER2-expressing tumors: preclinical assessment. *Journal of nuclear medicine: official publication, society of nuclear medicine*, 55 (11), 1842–1848
- Barui, S., et al., 2014. Simultaneous delivery of doxorubicin and curcumin encapsulated in liposomes of pegylated RGDK-lipopeptide to tumor vasculature. *Biomaterials*, 35 (5), 1643–1656
- Catania, A., et al., 2013. Immunoliposome encapsulation increases cytotoxic activity and selectivity of curcumin and resveratrol against HER2 overexpressing human breast cancer cells. *Breast cancer research and treatment*, 141 (1), 55–65
- Chen, Y., et al., 2012. Preparation of curcumin-loaded liposomes and evaluation of their skin permeation and pharmacodynamics. *Molecules*, 17 (5), 5972–5987
- Dhule, S.S., et al., 2012. Curcumin-loaded γ -cyclodextrin liposomal nanoparticles as delivery vehicles for osteosarcoma. *Nanomedicine: nanotechnology, biology, and medicine*, 8, 440–451.
- Eslaminejad, T., Ansari, M., and Eslaminejad, T., 2012a. Evaluation of the Potential of Trichoderma viride in the Control of Fungal Pathogens of Roselle (Hibiscus sabdariffa) in vitro. *Microbial pathogenesis*, 52 (4), 201–205
- Eslaminejad, T., Zakaria, M., and Eslaminejad, T., 2012b. Anti-fungal activity of cold and hot water extracts of spices against fungal pathogens of Roselle (Hibiscus sabdariffa) in vitro. *Microbial pathogenesis*, 52 (2), 125–129
- Feng, T., et al., 2017. Liposomal curcumin and its application in cancer. *International journal of nanomedicine*, 12, 6027–6044
- Filippi, A., et al., 2019. Anticancer effects of curcumin in luminal B and HER2 breast cancer cell line models. *Romanian biotechnological letters*, 24 (1), 168–175
- Fulda, S., 2010. Modulation of apoptosis by natural products for cancer therapy. *Planta medica*, 76 (11), 1075–1079
- Ghanemi, M., et al., 2018. Specific Targeting of HER2-Positive Head and Neck Squamous Cell Carcinoma Line HN5 by Idarubicin-ZHER2 Affibody Conjugate. *Current cancer drug targets*, 19 (1), 65–73
- Hasan, M., et al., 2014. Liposome encapsulation of curcumin: physico-chemical characterizations and effects on MCF7 cancer cell proliferation. *International journal of pharmaceuticals*, 461 (1–2), 519–528
- Ibrahim, S., et al., 2018. Curcumin marinosomes as promising nano-drug delivery system for lung cancer. *International journal of pharmaceuticals*, 540 (1–2), 40–49
- Klibanov, A.L., et al., 1991. Activity of amphipathic poly (ethylene glycol) 5000 to prolong the circulation time of liposomes depends on the liposome size and is unfavorable for immunoliposome binding to target. *Biochimica et biophysica acta (bba) - biomembranes*, 1062 (2), 142–148
- Lee, S.B., et al., 2008. Affibody molecules for in vivo characterization of HER2-positive tumors by near-infrared imaging. *Clinical cancer research: an official journal of the american association for cancer research*, 14 (12), 3840–3849
- Löfblom, J., et al., 2010. Affibody molecules: engineered proteins for therapeutic, diagnostic and biotechnological applications. *FEBS letters*, 584 (12), 2670–2680
- Ohadi, M., et al., 2017. Antioxidant potential and wound healing activity of biosurfactant produced by Acinetobacter junii B6. *Current pharmaceutical biotechnology*, 18, 900–908.
- Ranjan, A.P., et al., 2013. Efficacy of liposomal curcumin in a human pancreatic tumor xenograft model: inhibition of tumor growth and angiogenesis. *Anticancer research*, 33, 3603–3609
- Salehiabar, M., et al., 2018. Production of biological nanoparticles from bovine serum albumin as controlled release carrier for curcumin delivery. *International journal of biological macromolecules*, 115, 83–89.
- Santosh, M., et al., 2012. Paleoproterozoic ultrahigh-temperature granulites in the North China Craton: implications for tectonic models on extreme crustal metamorphism. *Precambrian research*, 222, 77–106
- Serwotka-Suszczak, A.M., et al., 2017. A conjugate based on anti-HER2 diaffibody and auristatin E targets HER2-positive cancer cells. *International journal of molecular sciences*, 18 (2), 401
- Shanmugam, M.K., et al., 2015. The multifaceted role of curcumin in cancer prevention and treatment. *Molecules*, 20 (2), 2728–2769
- Sochaj-Gregorczyk, A.M., et al., 2017. Design and in vitro evaluation of a cytotoxic conjugate based on the anti-HER2 affibody fused to the Fc fragment of IgG1. *International journal of molecular sciences*, 18 (8), 1688.
- Trousil, S., et al., 2014. Positron emission tomography imaging with 18F-labeled ZHER2: 2891 affibody for detection of HER2 expression and pharmacodynamic response to HER2-modulating therapies. *Clinical cancer research*, 20 (6), 1632–1643
- Yan, M., et al., 2015. HER2 expression status in diverse cancers: review of results from 37,992 patients. *Cancer metastasis reviews*, 34 (1), 157–164
- Zahmatkeshan, M., et al., 2016. Improved drug delivery and therapeutic efficacy of PEGylated liposomal doxorubicin by targeting anti-HER2 peptide in murine breast tumor model. *European journal of pharmaceutical sciences: official journal of the European federation for pharmaceutical sciences*, 86, 125–135
- Zokaei, E., et al., 2019. Therapeutic potential of DNase loaded on chitosan/cyclodextrin nanoparticle to recovery of chemosensitivity in the MCF-7 cell line. *Applied biochemistry and biotechnology*, 187, 708–723.