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Original Article

CURCUMIN-LOADED MULTI-VALENT LIGANDS CONJUGATED-NANOPARTICLES FOR ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT

Objective: The present study aims to develop curcumin-loaded muti-valent ligands conjugated-nanoparticlesfor targeted cell and study the biological activities including anti-inflammatory activity and kinetic cellular uptake.

Methods: Curcumin encapsulated PLGA nanoparticles were formulated by solvent displacement method. The cIBR and cLABL peptides were conjugated on the surface of PLGA nanoparticles using carbodiimide reaction to produce curcumin encapsulated cIBR-cLABL-nanoparticles (cIBR-cLABL-NPs). The expression of LFA-1 and ICAM-1 on the membrane of U937 cells were determined by flow cytometry. Kinetic binding and internalization profile of cIBR-cLABL-NPs and untargeted nanoparticles were also investigated by flow cytometry. Safety profile on PBMC and cytotoxicity profile on U937 cells were evaluated using the MTT assay. Anti-inflammatory activity was determined using aprotein denaturation method.

Results: The densities of cIBR and cLABL peptide on the surface of PLGA nanoparticles were 22.0±4.4 and 19.6±2.8 pmol/cm^2, respectively. The particle size and total surface area of PLGA were 256.1 nm and 0.038 m^2/g. The result showed that U937 cells expressed both LFA-1 and ICAM-1 proteins on their membranes indicating the possibility to use U937 cells as a targeted cell. According to the kinetic binding and internalization profiles, the cellular uptake of cIBR-cLABL-NPs was significantly higher than that of untargeted-NPs at all-time points indicating the specific uptake of cIBR-cLABL-NPs to target cell. Moreover, the rate of binding and internalization interpreted by the slope of linear regression of cIBR-cLABL-NPs was more rapid than that of untargeted-NPs. The MTT assay revealed the safety on human PBMC of cIBR-cLABL-NPs. The IC_{50} of free curcumin and curcumin-loaded cIBR-cLABL-NPs were 0.13 and 2.27 µg/ml, respectively. Protein denaturation assay presented the concentration dependence inhibition of protein denaturation by curcumin and cIBR-cLABL-NPs indicating anti-inflammatory activities of free curcumin and encapsulated curcumin.

Conclusion: cIBR-cLABL-nanoparicles increased the quantity of binding, rate of binding, and the internalization by target cells with safety profile on human PBMC. Moreover, the biological activity of encapsulating agent was maintained. Therefore, it could be used as a drug delivery system for encapsulating anti-infalmmatory agents.

Keywords: Curcumin, Multi-valent ligands, Anti-inflammation, Polymeric nanoparticles, cIBR, cLABL.

INTRODUCTION

The turmeric plant, *Curcuma longa* Linn, is a member of the ginger family of herb (Zingiberaceae). The dried rhizome of turmeric is a rich source of phenolic compound named the curcuminoids. Three main members of curcuminoids are curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Curcumin was first isolated in 1815 by Vogel and was isolated in crystalline formas diferuloyl methane in 1870 by Daube[1]. Curcumin is an orange/yellow crystalline powder practically insoluble in water and ether but soluble in ethanol, dimethylsulfoxide, and acetone. Curcumin has been shown to suppress transformation, proliferation, and metastasis of tumors. These effects are mediated through its regulation of various transcription factors, growth factors, inflammatory cytokines, protein kinases, and other enzymes [1]. However, curcumin has a limitation of use due to poor absorption, rapid metabolism, rapid systemic elimination, heat labile, poor solubility, and photosensitivity [2].

In recent years, the active targeting carrier has had a dramatic impact on the development of the drug carrier due to it provides the better pharmacokinetic, pharmacodynamics, controlled and sustained release profiles of the drug, in particular, a lower systemic toxicity [3]. It usually employs surface conjugation of the nanoparticles with the targeting moiety, having a preferential affinity for surface receptors which are expressed uniquely or highly in targeted cells as compared to normal cells [4]. Normally, single ligand always is attached on drug carrier's surface to enhance the specific of binding but the multi-valent ligands have been shown to exhibit higher binding avidity and affinity for cell expressing all targeting proteins and only minimal binding to cells that express none or some target compared with the monomeric ligand [5]. Multi-valent ligands allow the drug delivery system to bind multiple receptors on targeting cell, which lead to the increase of affinity, strength, and stability of conjugated drug delivery system [6].

In 2003, Siahaan et al. generated the cyclic peptides (cIBL, cIBR, cIBC, CH4 and CH7) derived from residues 1-21 of ICAM-1 Domain 1 (N terminus), which were investigated for the inhibition of ICAM-1/lFA-1 interaction between T-cells and epithelial cells [7]. cIBR peptide showed the highest affinity to the isolated LFA-1 receptor compared to cIBC and cIBL[8] and also showed the strongest inhibition for T cell adhesion via inhibiting the LFA-1/ICAM-1 interaction [7]. In 2009, cIBR peptides were coupled to PLGA nanoparticles for targeting lymphoblastic leukemia T-cells [9].

The cLABL peptides were derived from the I-domain of the alpha subunit which is the binding site of ICAM-1 [10]. The cLABL peptides were found to inhibit homotypic and heterotypic T-cell adhesion to epithelial and endothelial monolayer [10]. The cLABL peptides were also found to bind domain-1 (D1) of ICAM-1 and ICAM-3 on T-cells as epithelial cell monolayer. The cLABL peptide was found to have a stable conformation by NMR and CD. Interestingly, the cLABL peptide can be internalized by ICAM-1 into the cytoplasmic domain [11]. The cLABL peptide was found to inhibit adhesion in a temperature and concentration dependent manner [12].

Take the advantages with the encapsulation of curcumin in nanoparticles for prolonging the activity and the coupling both cIBR and cLABL peptides on the surfaces of nanoparticles may be

explored as a way of enhancing ability of binding and overall efficiency of drug delivery system.

MATERIALS AND METHODS

Chemical materials

3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma–Aldrich (St. Louis, MO, USA). cIBR peptide (cyclo(1, 12)-Pen-PRGGSVLVTGC-OH)(Mw 1, 174.5) and cLABK peptide were synthesized on a Pioneer peptide synthesizer (Per Septive Biosystems, CA). Poly (DL-lactic co glycolic acid) (50:50) with terminal carboxylate group (PLGA, inherent viscosity 0.67 dL/g, Mw ~90 kDa) was purchased from LACTEL Absorbable Polymers International (Pelham, AL, USA). Pluronic F-127 was purchased from OBASF The chemical company (St. Louis, MO, USA). 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), and Phorbol12-myristate13-acetate (PMA) were purchased from Sigma Aldrich. (St. Louis, MO, USA). Coumarin-6 was obtained from Polysciences, Inc. (Warrington, PA, USA). RPMI 1640 medium was purchased from Invitrogen Cooperation (Grand Island, NY, USA). Bovine serum albumin (BSA) was purchased from FlukaBiochemika (St. Louis, MO, USA). Tris base was purchased from Fisher Chem Alert (Fair Lawn, NJ, USA). Mannitol was obtained from May and Beaker LTD. (Dagenham, Essex RM10 7XS, UK). Dialysis membrane (MWCO 12, 000-14, 000) was purchased from Membrane Filtration Products Inc. (Seguin, Texas, USA). Molt-3 cells were obtained from American Type Culture Collection (Manassas, VA, USA).

Monoclonal anti-human CD11 (anti LFA-1) was purchased from Ancell (Bayport, MN, USA). Rabbit polyclonal anti CD11a was purchased from Abcam (Cambridge, UK). Horseradish peroxidase (HRP)-conjugated sheep anti-rabbit IgG antibody was obtained from Promega (Madison, WI, USA). Super Signal West Pico Chemiluminescent was purchased from Pierce (Rockford, IL, USA). Polyvinylidine fluoride transfer membrane was purchased from Pall Corporation (Pensacola, FL, USA). Prestained protein ladder was purchased from Fermentas (Hanover, MD, USA). ICMA-1 antibody was purchased from Santa Cruz Biotechnology (Oregon, USA). Lymphoprep™ was purchased from Axis-Shield Po CAS (Rodeløkka, Oslo, Norway).

Preparation of Nanoparticles

Coumarin-6-loaded Nanoparticles

To determine the quantity of cellular uptake of multi-valent ligands conjugated-nanoparticle, coumarin-6 was entrapped in nanopaticles as the fluorescent marker for flow cytometry. Nanoparticles (NPs) encapsulating coumarin-6 was prepared using the solvent displacement method [9]. Coumarin 6 (0.05% w/v) and 40 mg PLGA were dissolved in 3 ml of acetone and sonicated for 15 min. This mixture was slowly dropped (17.6 ml/h) into the stirred aqueous solution containing 0.1% Pluronic® F-127-COOH (25 ml), anionic surfactant. Carboxylated Pluronic® F-127 was synthesized from hydroxylated Pluronic® F-127 according to the reported procedure [13].

Conjugation of multi-valent ligands on the surfaces of nanoparticles

Pluronic®F-127-COOH coated PLGA nanoparticles were buffered using 2-(N-morpholino) ethanesulfonic acid (MES; pH 6.5). Nanoparticles were incubated with 100 mM 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) and 50 mM N-hydroxysulfosuccinimide (sulfo-NHS) for 15 min. The activated carboxyl terminus of Pluronic® F127-COOH on the surface of nanoparticles were allowed to react with the amino terminus of the cIBR, cLABL, and the mixture of cIBR and cLABL peptides at least 12 hr at room temperature. Conjugated nanoparticles were collected by centrifugation (16, 089 g, 10 min) and were washed three times with purified water.

Characterization of nanoparticles

Size and charge

The size and charge of formulated nanoparticles were characterized using dynamic light scattering.

Evaluation of cIBR peptides and cLABL peptides on the surface of nanoparticles

To determine the density of the cIBR, cLABL, and the mixture of cIBR and cLABL peptides on all PLGA-NPs, the amount of uncoupled peptides were quantified by a gradient reverse phase HPLC using C-18 column. Gradient elution was carried out at a constant flow of 1 ml/min, from 100% acetonitrile-water (5:95) with 0.1% trifluoroacetic acid to 0% acetonitrile-water (5:95) with 0.1% trifluoroacetic acid (corresponding to 0% acetonitrile–water (90:10, v/v) with 0.1% TFA to 100% acetonitrile–water (90:10, v/v) with 0.1% TFA for 15 min, following by an isocratic elution at 100% acetonitrile–water (90:10, v/v) with 0.1% TFA for 3 min. The densities of peptides on the surfaces of nanoparticles were calculated from the total surface area assuming a normal Guassian particle size distribution [12, 14].

Cell line

U937, LFA-1 and ICAM-1 expressing cell line, was cultured in suspension in RPMI 1640 media supplemented with 10% (v/v) fetal bovine serum and 1%(v/v) penicillin-streptomycin (10, 000 U/ml), in humidified 37 ˚C incubator with 5% CO2 atmosphere.

Expression of LFA-1 and ICAM-1 on U937 membrane

The expressions of LFA-1 and ICAM-1 on U937 cells were quantified in this study. Cells ($2x10^6$ cells/ml) were incubated AB serum (25 µl) at 4 °C for 10 min to block non–specific binding. Cells were reacted with anti LFA-1 (0.1 mg/ml) or anti-ICAM-1 ($1µg/1x10^6$ cells) antibody at 4 °C for 45 min. Free antibodies were removed by centrifugation. Finally, cells were fixed with 4% para formaldehyde. The fluorescent intensity was determined using a flow cytometer.

In vitro cellular uptake

To study quantitative cellular uptake of formulated-nanoparticles, LFA-1 and ICAM-1 expressing cell line was seeded in 96-well plates which contained $CaCl_2$ (1.5 mM) at a density of $2x10^5$ cells per well in serum free medium. Cells were incubated with cIBR-cLABL-nanoparticles or untargeted nanoparticles encapsulating coumarin-6 at the concentration of 2.2 mg/ml at 37 °C for 60 min. Cells were pelleted by centrifugation (460g, 4 °C for 2 min) and were washed three times with cold 0.1% BSA in PBS to eliminate excess particles and were fixed with 4% para formaldehyde before measuring the fluorescent intensity by flow cytometer.

Cytotoxicity assay

To determine the effect of cIBR-cLABL-nanoparticles encapsulating curcumin on cell viability, *in vitro* cytotoxicity of U937cells and PBMC were evaluated by MTT assay.

PBMC isolation

Peripheral blood sample was taken from a healthy volunteer into sterile, pyrogen-free disposable syringes with endotoxin-free heparin (200 i. u./u. i./40 µl). The blood sample was diluted with the same volume of phosphate buffer saline (PBS). Isolation of PBMCs from the subjects was performed using standard density gradient centrifugation In brief, PBMC was washed, resuspended and cultured in serum-free RPMI 1640 containing 0.5% L-glutamine and 1% penicillin-streptomycin.

Cell viability assay

Cells were seeded at a density of $5×10^5$ cells/well in a 96-wellsplate. Following 24 h incubation, cells were treated with several concentrations of all formulated nanoparticles for 48 h. MTT (3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyltetrazolium bromide stock solution (5 mg/ml) were added and the cells were incubated for 4 h. The medium were removed, 100 µl of dimethyl sulfoxide (DMSO) was added to dissolve the MTT crystals and the optical density was read using amicroplate reader with 540 nm as theexcitation wavelength and 650 nm as the background.

Assessment of *in vitro* anti-inflammatory activity: Inhibition of albumin denaturation

Method of *Mizushima et al.* [15] was followed with minor modifications. The reaction mixture was consisting of free curcumin

or curcumin-loaeded nanoparticles (cur-NPs) or curcumin-loaded cIBR-cLABL conjugated nanoparticles (cur-cIBR-cLABL-NPs) or indomethacin (positive control) and 1% aqueous solution of bovine albumin fraction. pH of the reaction mixture was adjusted using small amount of 1N HCl. Stock solutions of samples were prepared by using PBS (pH 7.4) as a solvent. Fifty microliters of each sample was transferred to microcentrifuge tubes and 500 µl of 1 % w/v BSA was added to all the above microcentrifuge tubes. The control consists of 500 µl of 1 % w/v BSA solution with 50 µl PBS. The standard consist 100 µg/ml of indomethacin in methanol with 500 µl of 1 % w/v BSA solution. The samples were incubated at 37°C for 20 min and then heated at 57°C for 20 min. After cooling the samples, the turbidity was measured spectrophotometrically at 660 nm. The experiment was performed in triplicate. The % inhibition of precipitation (denaturation of the protein) was determined on a % basis relative to the control using the following formula:

$$\% \text{ Inhibition} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) \times 100}{\text{Abs}_{\text{control}}}$$

Statistical analysis

Statistical evaluation of data was performed using an analysis of variance (one-way ANOVA). Newman–Keuls was used as a post-hoc test to assess the significance of differences. To compare the significance of the difference between the means of two groups, a t-test was performed. In all cases, a value of $p<0.05$ was accepted as significant.

RESULTS AND DISCUSSION

Physicochemical properties of nanoparticles

To observe the specific intracellular delivery of multi-valent ligands conjugated-nanoparticles, coumarin-6 was encapsulated in PLGA nanoparticles and used as a fluorescence marker for the detection of binding and internalization by U937 cells. PLGA nanoparticles were prepared by a solvent displacement method and the mixture of cIBR and cLABL peptides were conjugated on the surfaces of nanoparticles by carbodiimide chemistry. The physiochemical properties of untargeted-NPs and cIBR-cLABL-NPs were characterized in table 1. The mean diameter of untargeted-NPs and cIBR-cLABL-NPs were 190.2±13.1 and 256.1±30.7 nm, respectively. Low polydispersity indexes were obtained from all nanoparticles indicating the narrow size distribution of nanoparticles suspension and homogenous systems. The zeta potentials of untargeted-NPs and cIBR-cLABL-NPs were-28.2±2.6 mV and-15.3±0.8 mV, respectively. This may be due to the masking of anionic surface charges by Pluronic F-127 COOH coating. The physiochemical properties of drug carriers are the essential factors to predict the fate of the system after administration. Particle size is an important parameter as it can directly affect the physical stability, cellular uptake, bio distribution, and drug release from the nanoparticles [16]. The modifying surface characteristic can prevent particles uptake by the reticuloendothelial system (RES) but cannot assist nanoparticles to deliver exactly to the targeted site.

Table 1: Physicochemical properties of nanoparticles

	Effective diameter (nm) [mean±S. D.]	PDI [mean±S. D.]	Zeta potential (mV) [mean±S. D.]
Untargeted NPs	190.2±13.1	0.09±0.08	-28.2±2.6
cIBR-cLABL NPs	256.1±30.7	0.15±0.06	-15.3±0.8

Values are representative of three experiments (mean±SD).

Densities of peptides on the surface of nanoparticles

Active targeting was employed to assist nanoparticles by attaching the targeting ligand to the nanopartice surface. In this study, the carbodiimide reaction was used in the covalent conjugation between the cIBR peptide and carboxylic group on the nanoparticles surface. The carboxyl group of Pluronic F-127 COOH was converted to an active carboxyl form by carbodiimide reagents (EDC and NHS). Active carboxyl groups on nanoparticles' surfaces were coupled with free amino groups in cIBR and cLABL peptides to form amide bonds for this conjugation. After the reaction, free cIBR and cLABL peptides were collected and assayed using HPLC-UV to determine density of peptides on the surface of nanoparticles. It was calculated by assuming a normal Gussian particles size distribution. Effective diameter, total surface area, and surface cIBR of cIBR-cLABL NPs are showed in table 2.

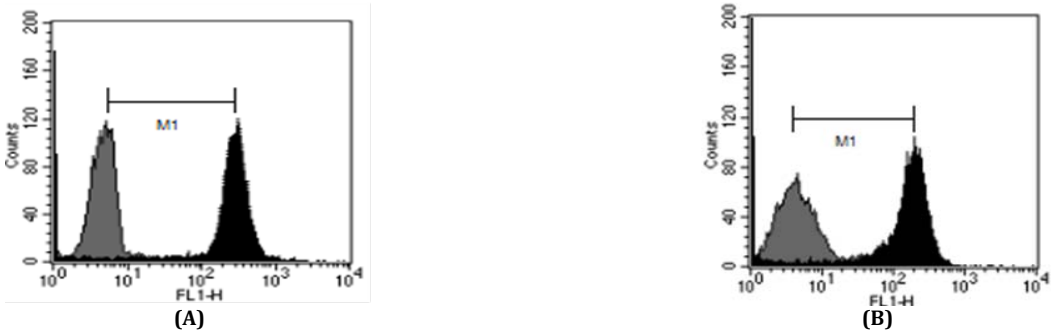
The expressions of LFA-1 and ICAM-1 proteins on U937 cell membrane

The expressions of LFA-1 and ICAM-1 proteins on U937 cell membrane were determined by flow cytometry. From the histogram, the results showed that U937 cell expressed both of LFA-1 and ICAM-1 as show in Fig.1. These results indicated that U937 cell can be used as the targeted cell for cIBR-cLABL-NPs due to the co-expressing of ICAM-1 and LFA-1 on thecell membrane.

Table 2: Densities of peptides on the surface of nanoparticles

	Diameter (nm)	Total surface area [m^2/g of PLGA]	Surface cIBR [pmol/cm^2]	Surface cLABL [pmol/cm^2]
cIBR-cLABL NPs	256.1	0.038	22.0±4.4	19.6±2.8

Values are representative of three experiments (mean±SD).



(A) **(B)**

Fig. 1: Histogram of the expression of ICMA-1 (A) and LFA-1(B) on U937 membrane

Binding and internalization of nanoparticles

The binding and internalization of cIBR-cLABL-NPS and untargeted-NPS are shown in fig. 2. Cellular uptake of cIBR-cLABL-NPs was significantly higher than that of untargeted-NPs at all-time points indicating the specific uptake of cIBR-cLABL-NPs to target cell. The greater amount of cIBR-cLABL-NPs in the cells indicated the enhanced internalization and retention effect of the targeted carrier via ligand-receptor endocytosis, leading cIBR-cLABL-NPs to beabetter target than nanoparticles without cIBR and cLABL conjugation. Without targeting ligand, the untargeted NPs could enter the cells and diffuse away from the cells over time. The fluorescent intensities of U937 cells after 5, 30, and 60 min of incubation, with cIBR-cLABL-NPs were higher than those of untargeted-NPs by ~1.5, ~1.8, and ~2.2-fold, respectively. The results indicated that the uptake activity of cIBR-cLABL-NPs was more rapid than that of untargeted-NPs. The results were in good agreement with the slope of linear regression lines as showed in fig. 3 since the velocity of binding and internalization could be determined by means of slope. The slope of cIBR-cLABL-NPs binding and internalization was 1.317 which was higher than that of untargeted-NPs, supporting that the binding and internalization of cIBR-cLABL-NPs was more rapid than that of untargeted-NPs.

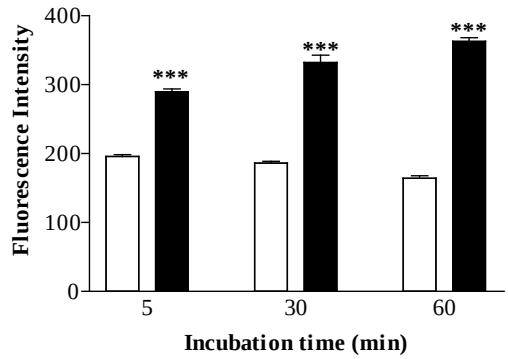


Fig. 2: Binding and internalization of cIBR-cLABL-NPs (■) and untargeted-NPs (□) after incubation with U937 cell. Results are given as mean±S. D. (n=10); * indicates *p<0.01***

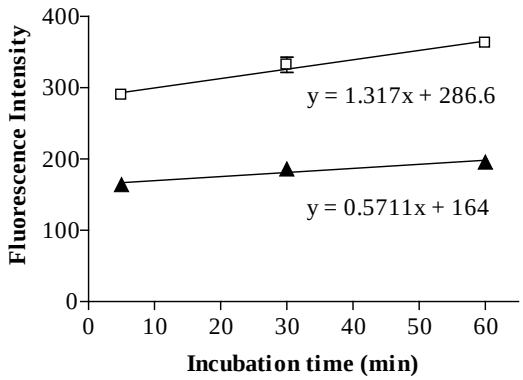


Fig. 3: Linear regression lines of cIBR-cLABL-NPs (□) and untargeted-NPs (▲) binding profiles by U937 cell

Cytotoxicity of free curcumin and cIBR-cLABL-NPs to U937 cells

Developing a drug delivery system which is uniform, biocompatible, and non-toxic, is a challenge issue in drug delivery [17]. Thus, the cytotoxicity effects of free curcumin and curcumin-loaded cIBR-cLABL-NPs were conducted by an established MTT assay. The cytotoxic effect of curcumin-loaded cIBR-cLABL-NPs was investigated in comparison with free curcumin when the concentration of curcumin ranged from 0.18 to 5.4 µg/ml. Curcumin-loaded cIBR-cLABL-NPs showed no significant cytotoxicity to PBMCs (fig. 4) in all different concentrations (0.18-5.4 µg/ml) leading curcumin-loaded cIBR-cLABL-NPs systems to be biocompatible. IC_{50} of free curcumin and curcumin-loaded cIBR-cLABL-NPs were 0.13 and 2.27 µg/ml, respectively (fig. 5). However, it can be seen that the cytotoxicity of curcumin-loaded cIBR-cLABL-NPs was lower than that of the free curcumin. This may be explained by the uptake of free curcumin was diffusion mechanism, while curcumin-loaded cIBR-cLABL-NPs was taken up into the cells via receptor-mediated endocytosis which involved the concentration of specific plasma membrane protein to the clathrin-coated pits, followed by the internalization and delivery to early endosomes [18]. Therefore, the rate of uptake via receptor-endocytosis pathway will depend on the amount of free receptor for ligand binding leading to the lower rate of uptake compared to thediffusion rate of free curcumin[19].

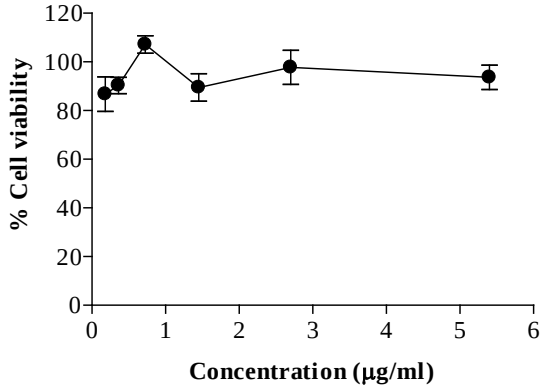


Fig. 4: Safety profile of curcumin-loaded cIBR-cLABL-NPs on PBMCs measured by the MTT assay. Results are represented in triplicate.

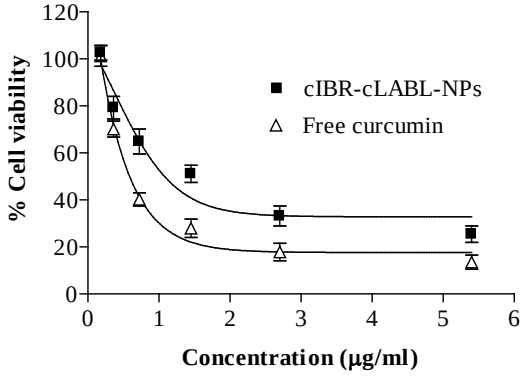


Fig. 5: Cytotoxicity results of free curcumin and curcumin-loaded cIBR-cLABL-NPs (cIBR-cLABL-NPs) to U937 cell. Results are represented in triplicate

Inhibition of albumin denaturation

Denaturation of tissue protein is one of known-documented causes of inflammatory and arthritic diseases. Production of auto antigens in certain arthritic diseases may be due to *in vivo* denaturation of proteins [20]. Therefore, protein denatured-preventing agents would be worthwhile for anti-inflammatory drug development [21]. In this study, the *in vitro* anti-inflammatory effect of free curcumin and curcumin-loaded cIBR-cLABL-NPs were evaluated against the denaturation of bovine serum albumin. The results from fig. 6 exhibited a concentration dependent inhibition of protein denaturation by curcumin and cIBR-cLABL-NPs. Literatures

suggested that the anti-denaturation of BSA was due to the presence of two interesting binding sites in the aromatic tyrosine rich and aliphatic threonine and lysine residue regions of BSA [22]. Compounds interacting with aliphatic regions around the lysine residue on the BSA could exhibit an anti-inflammatory activity such as polyphenol [23]. Therefore, curcumin could be an anti-inflammatory agent due to its phenolic structure. IC_{50} offree curcumin and curcumin-loaded cIBR-cLABL-NPs were 3.537 and 4.129 µg/ml, respectively. The effect of free curcumin on the inhibition of protein denaturation was more potent than that of cIBR-cLABL-NPs. This phenomenon may be explained bythe degradation profile of PLGA which leaded to the release of the encapsulated drug. The degradation of PLGA occurs through a reaction with water, and the rate of degradation depends on the availability of water molecules [24]. Thus curcumin in cIBR-cLABL-NPs may require sufficient time to be released in the medium before inhibiting the denaturation of protein. However, the effective protection of PLGA for natural polyphenolic compounds from the rising of temperature[25] may be an advantage point to consider PLGA nanoparticles as drug delivery system for heat-labile compound, including curcumin[26].

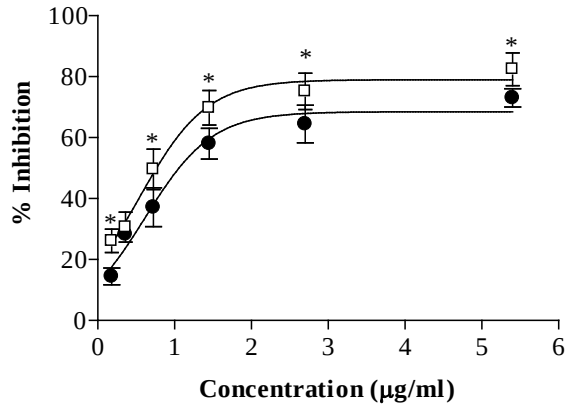


Fig. 6: Effect of free curcumin and cIBR-cLABL-cucumin-loaded nanoparticles (cIBR-cLABL-NPs) on inhibition of bovine serum albumin denaturation

CONCLUSION

Carbodiimide reaction was employed to conjugate the cIBR and cLABL peptides on the surface of PLGA nanoparticles which were formulated by solvent displacement method. The size of nanoparticles was increased from around 190 nm to 250 nm after conjugation. The conjugation leaded to zeta potential decrease because the consumption of COOH on the surface of nanoparticles by amino groups in cIBR and cLABL peptides. The results revealed that the binding and internalization of cIBR-cLABL-NPs were significantly higher than that of untargeted nanoparticles at all time points (~1.5, ~1.8, and ~2.2-fold, for 5, 30, and 60 min, respectively). Uptake activity of cIBR-cLABL-NPs was specific and occurred more rapidly than that of untargeted-NPs. Moreover, cIBR-cLABL-NPs still provided the cytotoxicity effect on U937 cells, safety profile on human PBMC, and anti-inflammatory activity of encapsulated curcumin. Inconclusion, the cIBR-cLABL-NPs would be an interesting drug delivery system for encapsulation of active agents, especially for the treatment of LFA-1 and ICAM-1 co-expression related diseases.

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CONFLICT OF INTERESTS

Declared None

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