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Protective effects of dendrosomal curcumin on an animal metastatic breast tumor

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ABSTRACT

Curcumin has been shown to inhibit migration and invasion of cancer angiogenesis *via* interacting with key regulatory molecules like NF-κB. Rapidly metabolized and conjugated in the liver, curcumin has the limited systemic bioavailability. Previous results have shown a new light of potential biocompatibility, biodegradability, as well as anti-cancer effects of dendrosomal curcumin (DNC) in biological systems. The present study aims to deliberate the protective effects of DNC on metastatic breast tumor *in vitro* and *in vivo*. After the dosing procedure, twenty-seven female mice were divided into 40 and 80 mg/kg groups of DNC, along with a control group to investigate the anti-metastatic effects of DNC on mammary tumor-bearing mice. *In vitro* results showed that the different concentrations of DNC reduced the migration and the adhesion of 4T1 cells after 24 h ($P<0.05$). Under the dosing procedure, DNC was safe at 80 mg/kg and lower doses. The treated DNC animals had a higher survival rate and lower metastatic signs (14%) compared to control (100%) ($P<0.05$). The metastatic tumors were more common in control mice than the treated groups in the lung, the liver and the sternum tissues. Animals treated with DNC had smaller tumor volume in comparison with control group ($P<0.05$). Final mean tumor volume reached to approximately 1.11, 0.31 and 0.27 cm^3 in the control, and 40 and 80 mg/kg DNC groups, respectively ($P<0.05$). Furthermore, suppression of NF-κB expression by DNC led to down-regulation of VEGF, COX-2, and MMP-9 expressions in the breast tumor, the lung, the brain, the spleen and the liver tissues ($P<0.05$). These outcomes indicate that dendrosomal curcumin has a chemoprotective effect on the breast cancer metastasis through suppression of NF-κB and its regulated gene products.

1. Introduction

Metastasis is a multi-step process involving complex interactions between the disseminating cancer cells, and their microenvironment (Alizadeh et al., 2014). Cancer metastasis is the cause of 90% of all deaths from cancer and exhibits an outstandingly different situation of clinical characteristics (Khan and Mukhtar, 2010), therefore, agents that inhibit metastasis provide a major advantage in treating cancers. Most tumors activate the transcription factor nuclear factor-kB (NF-kB), whereas natural chemopreventive agents suppress it, indicating a strong link between the tumor biology and the anti-cancer effects of various natural compounds (Luo et al., 2005). Experimental evidence has suggested that NF-κB has an important role not only in cancer initiation but also in cancer progression and metastasis (Huber et al., 2004). NF-κB regulates the genes expression involved in cancer metastasis such as MMPs, VEGF and COX-2 (Xie et al., 2010). NF-κB has also been described as a major culprit in cancer because it is constitutively activated in most human cancers, especially in the poorly differentiated cancers like those pertaining to the breasts (Bharti and Aggarwal, 2002). Several studies have shown that curcumin inhibits cancer angiogenesis, specifically migration and invasion through interacting with the key regulatory molecules like NF-κB (Himelstein et al., 1993).

Curcumin is a lipid-soluble compound extracted from the plant *Curcuma Longa*, and can potentially prevent cancer development

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with no discernible toxicity (Kelloff et al., 2000; Aggarwal et al., 2007). It is cost-effective, and has been used for centuries without known side-effects (Shishodia et al., 2005). However, absorption, distribution, metabolism and excretory studies of curcumin in recent years focused on its low bioavailability in systemic circulation (Ghalandarlaki et al., 2014). Subsequently, many methods were tested to overcome this defect like the use of the dendrosomal curcumin (DNC) (Sarbolouki et al., 2000; Alizadeh et al., 2012). Our previous results shed a new light on the potential biocompatibility, the biodegradability and the anticancer effects of DNC in the biological systems (Alizadeh et al., 2012; Babaei et al., 2012; Sarbolouki et al., 2012; Khaniki et al., 2013; Alizadeh et al., 2015; Mirgani et al., 2014). Accordingly, the present study has been designed to investigate the protective effects of DNC on the metastatic breast cancer cell line, and the model metastatic of mouse mammary tumor-bearing.

2. Materials and methods

2.1. Materials

Curcumin was purchased from Merck KGaA (Darmstadt, Germany) with a purity of 95%. The polymeric nanocarrier was locally produced in our lab (Patent Number: 71753). Methylthiazol tetrazolium (MTT), phosphate-buffered saline (PBS) solution, Ketamine and Xylazine were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin, streptomycin were from Life Technologies.

2.2. Dendrosomal curcumin preparation

For DNC preparation, the optimized protocol was used as previously described (Mirgani et al., 2014). Briefly, different w/w ratios of DNC ranging from 50:1 to 1:1 were examined before settling a suitable ratio of 7:1. Curcumin was dissolved in various amounts of dendrosome, and checked for absorbance spectra by UV spectrophotometery (TECAN, Switzerland). Appropriate mixture of dendrosome and curcumin were evaluated for the excitation/emission values in comparison with curcumin dissolved in PBS and 1% methanol as control. Briefly, curcumin and dendrosome were co-dissolved in 5 ml of acetone. The solution was then added into 5 ml of PBS and stirred. To evaporate acetone, the solution was finally left in a rotary evaporator. The dendrosome/curcumin micelle solution was sterilized using a 0.22 μm syringe filter (Millex-LG, Millipore Co. USA). Prepared DNC was stored in 4 °C in a light protected condition until used.

2.3. The study design

The present study was conducted in two series of experiments in order to obtain the protective effects of DNC (i) on the metastatic breast cancer cell line, and (ii) on the metastatic mouse mammary tumor-bearing.

2.4. Cell lines and their culture condition

The metastatic breast cancer cell line (4T1) and the normal mouse embryonic fibroblastic cells were procured from the national cell bank of Pasteur Institute, Tehran, Iran. The cells were cultured in Gibco® high glucose Dulbecco's Modified Eagle Medium. They were supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. All cells were grown at 37 °C in a humidified atmosphere of 5% carbon dioxide.

2.5. Cell viability assay

Cell viability assessed with methylthiazol tetrazolium (MTT) assay (Sigma-Aldrich) according to the manufacturer's instruction. Briefly, the cells were plated onto 96-well plates and incubated for 24, 48, and 72 h in the presence or absence of the different concentrations of DNC. Dendrosome alone was used to test the nanocarriers' cytotoxicity. Doxorubicin and void curcumin were used to evaluate the comparative toxicity of DNC on both cancerous and normal cell lines. Media containing the treatment agents were carefully removed afterwards, and the cells were washed twice with PBS. Having kept at 37 °C for 4 h, the medium was totally removed, and 200 μl dimethyl sulfoxide solution was added to each well. Absorbance which is directly proportional to the cell viability was subsequently measured at 570 nm in each well, and presented as the percentage of cell viability of treated cells against control cells using an enzyme-linked immunosorbent assay plate reader.

2.6. Scratch assay

Migration of 4T1 cells was measured by the scratch assay as previously described with some modifications (Oudhoff et al., 2008). The cells were cultured in 24-well plates and DMEM containing 10% FBS to nearly confluent cell monolayer, and a scratch wound was then created on the cell surface using a micropipette tip. The monolayer was once washed with PBS to remove debris or the detached cells from the monolayer. The cells were incubated at different concentrations (0, 5, 10, 15, and 20 μM) of DNC and void curcumin. The cultures were then incubated at 37 °C, subsequently photographed with microscope at 0 and 24 h. For each time point, four measurements per scratch were carried out. For the quantification and statistical analysis, the individual scratch width (micrometer, mean and standard deviation) was measured using the Image J software. The scratch area closed rate was measured for the different concentrations of DNC at 24 h post-treatment (the scratch width at 0 h was supposed 1 μm), and was calculated according to the following equation:

The percentage of the scratch area closed = (scratch width at 0 h − the remaining scratch width at 24 h)/scratch width in 0 h × 100%. The scratch area closed rate at 0 h in each group was treated as 0%. Experiments were performed in triplicate (Oudhoff et al., 2008).

2.7. Adhesion assay

To measure the relative attachment of 4T1 cells to immobilized fibronectin, 96-well plates were coated with 100 μl of 2.5 mg/ml fibronectin (Sigma, USA), and incubated overnight at 4 °C. Plates were then blocked with 100 μl PBS containing 3% (w/v) bovine serum albumin (BSA) for 30 min at 37 °C. To measure baseline nonspecific binding, other wells were coated with 1 mg/ml BSA. Following pretreatment of the cells with the different concentrations of DNC and void curcumin for 24 h, the cells were resuspended in serum-free DMEM and BSA (1:1) and incubated at 37 °C for 90 min to allow recovery of cell surface receptors and alleviate the effect of trypsin on the cells. Approximately 5×10^5 cells in 100 μl of DMEM-BSA were seeded in quadruplicates into each fibronectin-coated well, and incubated at 37 °C for 90 min. Nonadherent cells were removed by washing with PBS twice, and the adherent cells were fixed in ethanol for 10 min. After 5 min of crystal violet staining [0.1% (w/v) in 25% (v/v) methanol] at room temperature, the cells were gently rinsed with water five times to remove unbound stain and allowed to air-dry at room temperature. Fixed cells were lysed by 0.2% Triton X-100, and the absorbance was measured at 550 nm as follows: % Adhesion to matrix in 0 μM of DNC as 100 (Dastpeyman et al., 2012).

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