Molecular and cellular pharmacology

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 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 cm³ 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.

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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 Mulkhar, 2010), therefore, agents that inhibit metastasis provide a major advantage in treating cancers. Most tumors activate the transcription factor nuclear factor-kB (NF-κb), 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 through suppression of NF-κB and its regulated gene products.
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 of 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 spectrophotometry (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).
2.8. Animals

Animals have been handled according to relevant national and international guidelines of the Weatherall report, and Institutional Animal Care and Use Committee (IACUC) of Tehran University of Medical Sciences. Animals were housed in pens exceeding the stipulated size requirements. All inbred female BALB/c mice (6–8 weeks old, purchased from Iran Pasteur Institute) were maintained in large group houses under 12-h dark and light cycles and were given access to food and water ad libitum.

2.9. Dosing procedure

Twenty-seven BALB/c mice were equally divided to study the toxicity of DNC with doses of 320, 160, 80, 40, 20 and 10 mg/kg (B. W, i.p) for 7 consecutive days together control group. The dose with no adverse reactions during 24 h was assigned as a survival dose. Survived animals weighed on a daily basis and euthanatized one week later. Abnormal hematological/blood chemical indices, and the body weight changes were amongst the toxicity signs.

2.10. Hematology and blood chemistry tests

Animals decapitated under general anesthesia to evaluate the hematologic and the clinical chemistry parameters. Blood samples were taken and added into the ethylene-diamine-tetra-acetic-acid (EDTA)-coated tubes for hematology and heparin-coated tubes for the clinical chemistry tests. Total leukocyte count (WBC), erythrocyte count (RBC), platelets (Plt), hemoglobin (Hgb), and hematocrit (Hct) were measured by using an animal blood counter (Celltac; Nihon Kohden, Tokyo, Japan). Blood urea nitrogen (BUN), creatinine (Cr) and glucose (Glucose) were determined by using CCX System (CCX WB; Nova Biomedical, USA). Plasma alkaline phosphatase (ALP), albumin (ALB), alanine transaminase (ALT) and aspartate transaminase (AST) were also measured (Autoanalyser Model Biotecnica, BT 3500, Rome, Italy).

2.11. Tumorigenicity

4T1 cells were trypsinized and re-suspended in 10-fold excess culture medium. After centrifugation, cells were re-suspended in PBS, and 1 x 10⁶ cells were injected (0.1 ml, s.c) using a 21-gauge needle in the left flank of BALB/c mice under Ketamine and Xylazine (10 mg/kg, i.p) anesthesia. The tumors were seen after two weeks after cells injection.

2.12. Evaluation of DNC protective effects on metastatic mice breast tumor

According dosing procedure, twenty-seven mice were equally divided into three groups of 40 and 80 mg/kg doses of DNC, and control (saline) groups. DNC was given for 35 days after tumor injection from day 3 up to day 38. All animals euthanatized at day 42 post-cells injection. Tumor volume was measured on a weekly basis by a digital vernier caliper (Mitutoyo, Japan) and calculated using the following formula (Mohsenikia et al., 2013).

\[ V = \frac{1}{6} \pi L \times W \times D \]

where \( V \) = length, \( W \) = width, and \( D \) = depth.

2.13. Depiction of tumors and metastases

Animals were closely monitored for general health during the study period. Mice were weekly weighed, and observed for evidence of complicity and death. Moribund animals were killed. The rest were also euthanatized at the end of the 6th week. A thorough necropsy was then made, and vital organs including the liver, the spleen, the lung, the brain and the bone were examined for lesions and metastatic deposits. At the end of the 6th week, all animals underwent routine surgery and euthanasia. Weight, number, size, and location of the tumors were recorded.

2.14. Histological assay

The internal organs comprised of the liver, the spleen, the brain, the lung, the bone and the breast tumor were fixed in 10% formaldehyde, passed and embedded in paraffin. Paraflin blocks were then sectioned into 3–5 μm thickness for hematoxylin and eosin (H & E) staining. The slides were studied by OLYMPUS-BX51 microscope (Alizadeh et al., 2012). Digital photos were taken with OLYMPUS-DP12 camera and graded by the Scharff–Bloom–Richardson Scale (Mohsenikia et al., 2013).

2.15. RNA extraction and real-time polymerase chain reaction (PCR)

Total RNA was extracted from five different organs including the spleen, the liver, the brain, the lung and the tumor tissues using TRIzol™ reagent (Life Technologies) followed by DNase I digestion (Thermo Fisher Scientific, Waltham, MA, USA). Complementary DNA was synthesized by PrimeScript™ RT reagent kit (Fermentas, Germany). The list of primers for specific genes including NF-κB p65, VEGF, COX-2, MMP-9 and housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) indicated in Table 1. Real-time PCR was performed using the SYBR® Premix Ex Taq™ II (Takara). Relative gene expression was calculated as 2⁻ΔΔCt.

2.16. Statistical analysis

Statistics were presented in Prism® 6.1 software (GraphPad Software, Inc, La Jolla, CA, USA), and analyzed using one-way analysis of variance followed by Newman–Keuls multiple comparison test or two tailed student’s t-test. Incidences of tumors were compared by Fisher exact test. All data were expressed as mean ± S.D. Differences among groups were stated to be statistically significant when \( P < 0.05 \).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer (5’–3’)</th>
<th>Reverse primer (5’–3’)</th>
<th>Size (bp)</th>
</tr>
</thead>
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<tr>
<td>GAPDH</td>
<td>CACCAAGCCACTGACCAAG</td>
<td>TCGTATCCAGGACGACTAG</td>
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<tr>
<td>MMP9</td>
<td>TCCGATCCACAGAAGCAGCC</td>
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</tr>
<tr>
<td>VEGF</td>
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<td>GCCTTCGGTCGAGCAGAC</td>
<td>146</td>
</tr>
<tr>
<td>COX2</td>
<td>TGAACAGTCCAAGGAGCAAG</td>
<td>ACTAATGAGGACAGAATGAGC</td>
<td>200</td>
</tr>
<tr>
<td>NFκp105</td>
<td>TCTTACATTCGACATCCCC</td>
<td>CCAACCTCAGAAGCTCTCCT</td>
<td>178</td>
</tr>
</tbody>
</table>

Table 1: Nucleotide sequences of the primers used for real-time RT-PCR.
3. Results

3.1. DNC effects on cell viability

As shown in Fig. 1, DNC significantly suppressed the viability of 4T1 cells induced in a time- and dose dependent manner, half-maximal inhibitory concentration (IC50) of DNC for 4T1 cells was 32.5 and 25 μM after 24 h (Fig. 1A) and 48 h (Fig. 1B) respectively, which was declined to 17.5 μM at 72 h (Fig. 1C) (P < 0.001). However, the viability of 4T1 cells was affected by free curcumin only at 72 h (Fig. 1C). Curcumin and DNC had slight effects at higher concentrations on normal mouse embryonic fibroblast cells (Fig. 1D). No inhibitory effect was observed for void dendrosome.

3.2. Inhibitory effects of free curcumin and DNC on 4T1 cells migration

Free curcumin and DNC were able to significantly inhibit the migration of 4T1 cells in a dose dependent-manner after 24 h (Fig. 2). Compared to control, the migration of 4T1 cells was reduced by 5, 10, 15, and 20 μM free curcumin to 30% (P < 0.05),
3.3. Inhibitory effects of free curcumin and DNC on 4T1 cells adhesion

Free curcumin and DNC were able to significantly inhibit the adhesion of 4T1 cells to fibronectin in a dose-dependent manner after 24 h (Fig. 3). In comparison with control, the adhesion of 4T1 cells to matrix was reduced by 5, 10, 15, and 20 μM free curcumin to 35.5% (P < 0.05), 42% (P < 0.05), 46% (P < 0.05), and 71% (P < 0.05), respectively, and by 5, 10, 15, and 20 μM DNC to 42% (P < 0.05), 55% (P < 0.05), 65% (P < 0.05), and 78.5% (P < 0.05), respectively. Moreover, this reduction was significant by DNC in comparison with free curcumin at 15 and 20 μM concentrations (Fig. 2).

3.4. The DNC toxicity in mice

The main toxicity signs to DNC in various doses summarized in Table 2. The doses of 320 and 160 mg/kg of DNC associated with mild poisoning symptoms of hematological, hepatocellular and renal tissues, and DNC showed the remarkable safety rate up to 80 mg/kg (Table 2). Hematological markers were measured by a complete blood count analysis. No inflammatory responses were seen in treated groups since the total leukocyte counts remained within normal range (Table 2). A significant decrease in WBC was seen at 160 and 320 mg/kg DNC compared to control animals (P < 0.05) (Table 2).

BUN and Cr levels were measured for kidney function assessment, while ALP, ALB, ALT, and AST were done for liver function evaluation. BUN and Cr were significantly higher in animals of 160 and 320 mg/kg DNC compared to control animals (P < 0.05) (Table 2). Furthermore, the liver function tests showed the drastic increase in ALT and AST levels, and the decrease in ALB level in 160 and 320 mg/kg DNC compared to control mice (P < 0.05) (Table 2). It seems that the liver and the kidney were the target organs for DNC toxicity in the high doses.

3.5. Protective effects of DNC on metastatic mice breast tumor

3.5.1. General observations

Animals’ weight in the control and DNC treated groups slightly increased. Body weight rose steadily to reach a plateau after about 3 weeks in all animals, but showed a slight fall in the control group in the last few weeks as tumors developed (Fig. 4). Eight of the twenty-seven mice died 3–7 weeks after tumor injection in the control (n = 5), 40 mg/kg DNC (n = 2) and 80 mg/kg DNC groups.

![Fig. 4. A plot of dendrosomal curcumin effects on mice weight.](image-url)
DNC treated animals had higher survival rates compared to the control (Fig. 5). Except for a slight fall in food intake (g/day) in the control group, there were no significant differences in terms of the intake in control and treatment groups. No behavioral changes were observed in the animals during the course of administration, or in the ensuing follow up period.

3.6. Tumor type

Tumor cells developed with sarcomatoid-type areas showing morphological signs of epithelial differentiation into surrounding tissues. Tumor cell infiltration observed in the surrounding tissues and nests of carcinoma cells with grade II/III based on Scharff-Bloom-Richardson scale (Fig. 6).

3.7. Tumor incidence and size

At the end of the second week, the tumor take rate was 100% in control and treated groups. However, at the end of the study, mice in control group had higher tumor induction in comparison to the treated groups. Additionally, mice treated with DNC had smaller tumor volume (cm³) in comparison with control (Fig. 7A). The average tumor volume was significantly less in DNC groups than in control groups at 21, 28, 35 and 42 days after tumor cells injection (P < 0.05). The mean final tumor volume reached to approximately 1.11, 0.31 and 0.27 cm³ in the control, 40 and 80 mg/kg DNC groups, respectively (Fig. 7A). Moreover, the average final tumor weight reached to approximately 1400, 550 and 500 mg in the control, 40 and 80 mg/kg DNC groups, respectively (Fig. 7B).

3.8. Tumor metastases

At the end of the study, a thorough necropsy was made, and the vital organs including the liver, the spleen, the lung, the brain and the bone were examined for lesions and metastatic deposits. They were fixed in 10% formaldehyde, passaged and embedded in paraffin, and were then sectioned into 3–5 μm thickness for H & E staining. The slides were read by two evaluators using an OLYMPUS-BX51 microscope. Results showed that the incidence of the breast tumor metastases was about 89% in the control animals (n = 8) in comparison with about 11% in both 40 mg/kg (n = 1) and 80 mg/kg (n = 1) of DNC doses. Among the treatment groups, reduction in the tumor incidence in 40 and 80 mg/kg DNC ranged equally to 87.6% compared to control (P < 0.001). Furthermore, the number of metastases per mouse in the metastatic mice varied from one to three. The tumors were scattered in the different parts of the body. Metastatic tumors were more common in the lung (Fig. 8A), the liver (Fig. 8B) and the sternum tissue (Fig. 8C).

3.9. Analysis of gene expression by real-time PCR

NF-κB p105 mRNA expression was significantly down-regulated in 40 and 80 mg/kg of DNC compared to control in the breast tumor, the lung, the brain, the liver and the spleen tissues (P<0.05).
tumor cell line and a typical animal model of metastic breast cancer. The in vitro results indicated that DNC inhibited the migration and the adhesion of 4T1 cells. The in vivo analyses demonstrated a significant DNC-mediated reduction in the incidence, the size and the weight of tumors. The metastatic tumors were more common in the control than the treated groups in the lung, the liver and the sternum tissue. This was accompanied by suppression of the expression of NF-κB p105 and its downstream genes including VEGF, COX-2, and MMP9 in the breast tumor and in the lung, the brain, the spleen and the liver tissue. These findings imply the fact that DNC can act as an anti-metastatic agent.

The present study aimed the role of dendrosomal curcumin as an agent anti-metastasis, on the cell viability, the migration and the adhesion of 4T1 cells, which is the metastatic cancer cell line both in vitro and in vivo. The scratch and the adhesion assays are commonly used method to study the migratory and the adhesive behavior of cells that are fundamental to diverse biologic processes such as tumor metastasis. The effects of DNC on the migration ability and the adhesion of 4T1 cells to matrix were performed at the concentration range 15 μM, and determined by the scratch and the adhesion assays which its concentration was 1/2 concentration obtained from 24 h MTT assay. The concentration and time dependent inhibition of the cell viability, the migration and the adhesion by DNC can show its central role in the process of cancer metastasis.

In the dosing procedure, high doses of DNC (160 and 250 mg/kg) brought the poisoning symptoms of hematological, hepatocellular and renal tissues. WBC was drastically decreased at the high doses compared to the control. Higher Cr and BUN levels may be released from damaged muscular cells. Significant decrease in albumin level, together with increased ALT and AST in DNC-treated animals points to the hepatic effects of the high doses that may induce by nanocarrier metabolism. We are not sure about the reversibility of DNC toxicity since it is beyond the scope of the present study.

Metastatic process is often associated with up-regulated MMPs, loosened extracellular matrix for cancer cells evasion. MMPs especially MMP-9, is known to be involved in tumor angiogenesis; mainly through its matrix-degrading capacity (Kiuchi et al., 1993). Previous reports have indicated that MMP-9 expression is transcriptionally regulated through NF-κB elements within MMP-9 gene (Farina et al., 1999). Aggarwal (2004) also showed that NF-κB activation is an absolute requirement in MMP-9 up-regulation (Aggarwal, 2004). Results showed that treatment with curcumin is associated with a decrease in NF-κB and MMP-9 expression levels (Hong et al., 2006; Bachmeier et al., 2007; Killian et al., 2012). It has also been found that curcumin inhibits the migratory activity, the proliferative rate, the adhesion, and the invasion of breast cancer cells through down-regulating NF-κB p65 expression (Chiu and Su, 2009; Kang et al., 2009). Therefore, our results are in agreement with previous reports showing that dendrosomal curcumin can inhibit MMP-9 expression in the breast tumor, the lung, the brain, the spleen and the liver tissue.

In another portion of the present study, we showed that DNC down-regulated VEGF expression in the breast tumor, the lung, the brain, the spleen and the liver tissue. VEGF plays a key role in cancer biology and being involved in tumor neovascularization in response to increased demand of tumor cells for obtaining a variety of nutrients as well as oxygen (Ferrara, 1999). It has also been suggested that VEGF induction is interceded by NF-κB intracellular signaling pathway. Consequently, VEGF down-regulation – as shown here – can explain the anti-metastatic activities of DNC (Gasparini et al., 2005). Furthermore, it is well established that VEGF and MMPs are the essential factors in angiogenesis and cancer cells invasion (Himmelstein et al., 1993; Carmeliet, 2004), and curcumin can inhibit the tumor growth and suppress the angiogenesis through inhibition of MMP-9 and neovascularization (Perry et al., 2010).

4. Discussion

The major objective and purpose of our study was to assess the anti-metastatic effects of dendrosomal curcumin in a mouse breast
COX-2 has been implicated in carcinogenic processes (Kardeh et al., 2014). Malignant cells induced COX-2 over-expression has been shown to enhance the cellular invasion, the angiogenesis and regulate the anti-apoptotic defensive activities of the cells (Shiraga et al., 2002). These effects have shown to be reversed by the anti-inflammatory agents for example; curcumin (Huei-Chen et al., 1992; Khaniki et al., 2013). Additionally, the several lines of evidence indicated the critical role of COX-2 in carcinogenesis (Koide et al., 1999; Gómez-Hernández et al., 2006) which is mediated by NF-κB intracellular signaling pathway (Wang et al., 2008). Correspondingly, curcumin appears to exert its anti-angiogenic effect through inhibition of COX-2 expression (Khaniki et al., 2013). Furthermore, pre-clinical studies have shown that curcumin suppressed COX-2 activity through NF-κB kinase enzymes suppression (Plummer et al., 1999).

Moreover, curcumin administration noticeably decreased metastasis to the lung tissue, and suppressed COX-2 expression in a human xenograft model of breast cancer (Aggarwal et al., 2005). In this regard, our results are in agreement with the previous reports in which curcumin inhibited NF-κB regulated COX-2 and MMP-9 expression (Kitamura et al., 1996; Plummer et al., 1999).

Animal and clinical trials have shown that curcumin and its analogs may be target critical genes associated with the tumor angiogenesis and metastasis (Im Kim et al., 2008; Kronski et al., 2014). Curcumin has been shown to decrease the ability of paclitaxel-resistant breast cancer cells to form the lung metastases via suppression of NF-κB, COX2 and MMP-9 expression (Aggarwal et al., 2005). In addition, a 78% reduction in the tumor metastasis was observed in nude mice receiving both curcumin (100 mg/kg) and paclitaxel (7 mg/kg) compared to control group in a xenograft model of breast cancer (Kang et al., 2009). The first clinical trial of curcumin investigated the feasibility and tolerability of docetaxel and curcumin combination in patients with the advanced and metastatic breast cancer (Bayet-Robert et al., 2010). Based on the results from the previous studies, phase I/II/III clinical trials of curcumin alone or in combination with other chemotherapeutic agents are currently ongoing in patients with different types of cancer including breast cancer (Gupta et al., 2013).

Taken together, the results of the current study reveal that DNC is effective in suppressing the metastatic tumor growth both in vitro and in vivo. As well, DNC exerted its chemoprotective anti-metastatic effects through suppression of NF-κB and its regulated gene products. It seems that dendrosomal curcumin may provide a clinically useful tool for abolishing the activity of VEGF, MMP-9 and COX-2 in tumor cells.

Declaration of interest

The authors reported no conflicts of interest. The authors alone are responsible for the content of the paper.

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References


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