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Novel anti-angiogenic effects of aromatic-turmerone, essential oil isolated from spice turmeric

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ABSTRACT

Angiogenesis is essential for tumor growth and there is a continuing need for exploring new anti-angiogenic agents from natural products including herbs. Aromatic (Ar)-turmerone isolated from the rhizome of *Curcuma longa* Linn. (Turmeric) exhibits anti-tumor and immunomodulatory activities. In this study, the anti-angiogenic effects of Ar-turmerone were evaluated in human microvascular endothelial cells, zebrafish and Matrigel plugs mouse models. The data obtained indicate that Ar-turmerone treatment significantly inhibits the proliferation, tube formation and motility of HMEC-1 cells at non-cytotoxic concentrations (4.6–9.2 μM, $p < 0.05$). The mRNA expressions of metalloproteinase-2 and -9 as well as adhesion molecules could be down-regulated by Ar-turmerone at 18.4 μM ($p < 0.05$). In zebrafish model, the new blood vessel growth in embryos was significantly blocked by Ar-turmerone treatment (12.5–25 μg/mL medium). The bFGF-induced blood vessel formation in Matrigel plugs in C57BL/6 mice was suppressed by Ar-turmerone (25–50 μg/mL Matrigel). Thus, the *in vitro* and *in vivo* anti-angiogenic activities of Ar-turmerone were demonstrated for the first time. The findings suggest that such a component of turmeric essential oil has the potential to be further developed as an anti-angiogenic agent.

1. Introduction

Lung, colorectal, prostate and breast cancers account for the majority of cancer mortality all over the world (International Agency for Research on Cancer, 2012). Apart from standard modalities of surgery, chemotherapy, radiotherapy, the identification of new molecular targets for cancer growth as well as intervening angiogenesis and metastasis with naturally isolated agents is crucial for reducing the mortality of cancers. The concept of anti-angiogenesis is now an important component in cancer treatment and it is believed that blocking

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Abbreviations: bFGF, basic fibroblast growth factor; FBS, fetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HMEC-1, human microvascular endothelial cells; PBS, phosphate-buffered saline; VEGF, vascular endothelial growth factor

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angiogenesis could be a strategy to arrest tumor growth and metastasis (Carmeliet & Jain, 2000). In the last decades, several drugs targeting the tumor vasculature and inhibiting tumor angiogenesis have been discovered (Folkman, 2007), and some anti-angiogenic agents are approved for clinical use, such as humanized anti-VEGF-A antibody bevacizumab, tyrosine kinase inhibitors sorafenib and sunitinib (Weis & Cheresh, 2011).

Many chemopreventive molecules isolated from natural products including taxol (Avramis, Kwock, & Avramis, 2001), curcumin (Yoysungnoen, Wirachwong, Changtam, Suksamrarn, & Patumraj, 2008), deoxybouvardin (Yue et al., 2011), are also known to inhibit angiogenesis. Nevertheless, there is a continuing need for new anti-angiogenic drugs, especially from natural sources including herbs. Turmeric, the rhizome of the perennial herb *Curcuma longa* Linn. (family Zingiberaceae), has been used in India and China for centuries as both a spice and a medicine. The turmeric extract consists of 3–5% essential oil and 0.02–2.0% curcuminoids (Braga, Leal, Carvalho, & Meireles, 2003; Gopalan, Goto, Kodama, & Hirose, 2000). Most of the previous studies on *C. longa* focused on a bioactive curcuminoid, curcumin, which is shown to be anti-inflammatory, anti-tumor, anti-oxidant, apoptotic and tumor suppressive in cancer cells (Goel, Kunnumakkara, & Aggarwal, 2008; Prasad, Tyagi, & Aggarwal, 2014). Nevertheless, pharmacological studies have also demonstrated anti-inflammatory, anti-tumor, anti-oxidant activities of the essential oil (Bagad, Joseph, Bhaskaran, & Agarwal, 2013; Jayaprakasha, Jena, Negi, & Sakariah, 2002; Shi, Ku, & Tan, 2003) and curcumin-free turmeric (Aggarwal, Yuan, Li, & Gupta, 2013). In our previous studies, turmerones isolated from *C. longa* extract were shown to modulate the absorption of curcumin in intestinal Caco-2 cells *in vitro* (Yue et al., 2012), and aromatic-turmerone (Ar-turmerone) was shown to have immunostimulating activities in human peripheral blood mononuclear cells (Yue et al., 2010). The apoptotic (Cheng et al., 2012; Ji, Choi, Lee, & Lee, 2004), anti-platelet (Lee, 2006), anti-inflammatory (Park, Jin, Kim, Kim, & Lee, 2012), anti-invasion (Park, Kim, Kim, & Lee, 2012), anti-tumor (Kim, Suh, Lee, & Lee, 2013) and anti-dermatophytic (Jankasem, Wuthi-Udomlert, & Gritsanapan, 2013) effects of Ar-turmerone have also been reported.

Although turmeric extract containing curcumin (Pantazis et al., 2010) and curcumin-loaded nanoparticles (Ding et al., 2014) were shown to have anti-angiogenic activities, the effects of Ar-turmerone on angiogenesis have not been investigated. In the present study, we demonstrated for the first time that Ar-turmerone possessed anti-angiogenic effects in human endothelial cells HMEC-1 and the mRNA expressions of matrix metalloproteinases and adhesion molecules were altered by Ar-turmerone. Besides, *in vivo* studies showed that Ar-turmerone could reduce blood vessels' growth in zebrafish and matrigel plug mouse model.

2. Materials and methods

2.1. Chemicals and reagents

Dried rhizome of *C. longa* Linn. (turmeric, Fig. 1A) were purchased from a renowned supplier (Wing Hing Company) in Hong Kong. Regarding the authentication of the raw herb, both morphological and chemical authentications have been accomplished in accordance with the Chinese Pharmacopoeia 2010 (Chinese Pharmacopoeia Commission, 2010) as described in our previous study (Yue et al., 2010). The chemical profiles have been compared qualitatively using thin layer chromatography (TLC) with the reference herb provided by the National Institute for the Control of Pharmaceutical and Biological Products. Authenticated voucher specimen (Number: 2011–3353) was deposited in the museum of the Institute of Chinese Medicine, The Chinese University of Hong Kong.

The human microvascular endothelial cells (HMEC-1) were purchased from American Type Culture Collection (Manassas, VA, USA). Fetal bovine serum (FBS), penicillin–streptomycin, trypsin-ethylenediaminetetraacetic acid (EDTA), recombinant vascular endothelial growth factor (VEGF), Trizol, dNTP were obtained from Life Technologies (Grand Island, NY, USA). MCDB 131 medium, basic human fibroblast growth factor (bFGF), heparin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) and Drabkin's reagent were obtained from Sigma (St. Louis, MO, USA). Basement membrane matrix Matrigel (GFR) was from BD Biosciences (San Jose, CA, USA). [Methyl-^{3}H]-thymidine and unifilters were from PerkinElmer (Waltham, MA, USA). Primary antibodies for Western blot were purchased from Abcam (Cambridge, UK), while secondary horseradish peroxidase-conjugated antibodies were from Life Technologies. Real-time PCR reagent iTaq Fast SYBR Green Supermix was from Bio-Rad (Hong Kong) and QuantiFast SYBR Green RT-PCR kit from Qiagen (Hilden, Germany).

2.2. Isolation of Ar-turmerone from turmeric extract

The dried rhizome of *C. longa* (turmeric) was powdered. The powdered turmeric (142.8 g) was extracted under reflux with methanol for 1 h and the extraction was repeated once. Following filtration, the crude methanol extract was centrifuged to remove undissolved particles. The turmeric methanol extract (15.8 g) was partitioned with hexane and the fraction was subjected to silica gel column chromatography eluted with dichloromethane. The turmeric oil fraction was further separated by silica gel column chromatography eluted with hexane and ethyl acetate (500:8, v/v) to obtain aromatic-turmerone (Ar-turmerone), and turmerone plus atlantone mixtures. The mixtures were then subjected to C18 column chromatography eluted with 80% acetonitrile. Pure Ar-turmerone (0.456 g, Fig. 1B) was obtained and the identification was based on the ^{1}H and ^{13}C NMR spectral analysis and mass spectrometry as explained in a previous study (Yue et al., 2010). The percentage yield of Ar-turmerone from turmeric methanol extract was 2.9% (w/w).

2.3. Cell culture

The HMEC-1 cells were maintained in MCDB 131 medium containing 2 mM glutamine, 1 μg/mL hydrocortisone and 10 ng/mL epidermal growth factor. All of the media were supplemented with 10% (v/v) heat-inactivated FBS, 100 units/mL penicillin–streptomycin. The cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2. When the cells reached 80% confluence in culture flasks, trypsin-EDTA was used to remove the cells and the cells were used in experiments or reseeded in