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Antitumor and antimetastatic actions of xanthoangelol and 4-hydroxyderricin isolated from *Angelica keiskei* roots through the inhibited activation and differentiation of M2 macrophages

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This paper is dedicated to Dr. Maho Sumiyoshi, 42 years old, who passed away on December 11th, 2014, 50 days after the original submission of this article.

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ABSTRACT

Background: Tumor growth and metastasis have been closely associated with the M2 macrophage-induced activation of tumor-associated macrophages (TAMs).
Purpose: The antitumor and antimetastatic actions of xanthangelol and 4-hydroxyderricin on the role of M2 macrophages in the TAMs of highly metastatic osteosarcoma LM8-bearing mice have not yet been fully elucidated. In order to clarify the mechanisms underlying the antitumor and antimetastatic actions of the above chalcones, we performed *in vivo* and *in vitro* studies.
Study Design: The antitumor and antimetastatic actions of xanthoangelol and 4-hydroxyderricin were examined *in vivo* and the effects on M2 macrophage differentiation and activation were examined *in vitro*.
Methods: We examined the antitumor and antimetastatic effects of xanthoangelol and 4-hydroxyderricin on highly metastatic osteosarcoma LM8-bearing mice (*in vivo*). Further, we examined their effects on the differentiation of interleukin (IL)-4 plus IL-13-induced M2 macrophages and activation of IL-4 plus IL13-induced M2 macrophages (*in vitro*). We also investigated the expression and phosphorylation of signal transducer and activator of transcript 3 (Stat 3) in the differentiation process of M2-polarized macrophages (*in vitro*).
Results: Xanthoangelol or 4-hydroxyderricin (25 or 50 mg/kg, twice daily) inhibited tumor growth, metastasis to the lung and liver, and TAM expression in tumors. In addition, xanthoangelol (10, 25 or 50 μM) and 4-hydroxyderricin (5, 10, 25 or 50 μM) inhibited the production of IL-10 and monocyte chemoattractant protein (MCP)-1 in M2-polarized macrophages. This result indicated that xanthoangelol and 4-hydroxyderricin inhibited the activation of M2 macrophages. Furthermore, xanthoangelol (5–50 μM) inhibited the phosphorylation of Stat 3 without affecting the expression of the Stat 3 protein in the differentiation process of M2 macrophages, which indicated that these chalcones inhibited the differentiation of M2 macrophages.
Conclusion: These findings suggested that the antitumor and antimetastatic actions of xanthoangelol and 4-hydroxyderrcin might be attributed to the regulated activated TAMs through the inhibition of activation and differentiation of M2 macrophages in the tumor microenvironment.

Introduction

The roots of *Angelica keiskei* Koizumi (Umbelliferae) have been used as diuretic, laxative, analeptic, and lactagogue. Recent studies have reported that xanthoangelol [(*E*)-1-[3-[(*E*)-3,7-dimethylocta-2,6-dien-1-yl]-2,4-dihydroxyphemyl]-3-(4-hydroxyphenyl)prop-2-en-1-one] and 4-hydroxyderrcin [(*E*)-1-[2-hydroxy-4-methoxy-3-(3-methylbut-2-en-1-yl)phenyl]-3-(4-hydroxyphenyl) prop-2-en-1-one] (Fig. 1) isolated from *A. keiskei* roots exhibited anti-platelet actions (Son et al. 2014) as well as anti-inflammatory responses in RAW 264 macrophages (Yasuda et al. 2014) and human umbilical vein endothelial cells (Okura et al. 2011). It has been also reported that xanthoangelol and 4-hydroxyderricin inhibited the differentiation of preadipocytes to adipocytes (Zhang et al. 2013) and the biosynthesis of melanin (Arung et al. 2012). Furthermore, Akihisa et al. (2012) reported that the above chalcones displayed cytotoxic activities and antitumor-promoting effects. We previously reported that

Abbreviations: CDDP, cisplatin; HE, hematoxylin–eosin; IL, interleukin; LLC, Lewis lung carcinoma; MCP, monocyte chemoattractant protein; PBS, phosphate buffered saline; PVDF, polyvinylidene difluoride; Stat 3, signal transducer and activator of transcription 3; TAM, tumor-associated macrophage.

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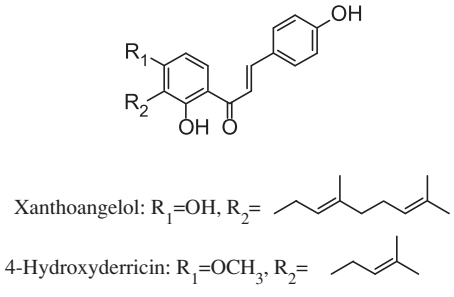

Fig. 1. Structures of xanthoangelol and 4-hydroxyderricin.

xanthoangelol and 4-hydroxyderricin exhibited antitumor and antimetastatic actions by inhibiting tumor-induced angiogenesis and/or the stimulation of immune function in Lewis lung carcinoma (LLC)-bearing mice (Kimura and Baba 2003; Kimura et al. 2004). Tumor-associated macrophages (TAMs) derived from circulating monocytes have been identified as the main components of the tumor microenvironment, and TAMs have consequently been shown to stimulate tumor growth and metastasis (Allavena and Mantovani 2012; Nakao et al. 2005; Schimieder et al. 2012). We also reported that tumor growth and metastasis may be stimulated by tumor-induced angiogenesis and lymphangiogenesis through increases in TAMs at the tumor site and blood monocyte chemoattractant protein-1 (MCP-1) (Kimura and Sumiyoshi 2013). This increase in TAMs around the tumor microenvironment has been closely associated with a poor prognosis in cancer patients (Joyce and Pollard 2009; Lewis and Pollard 2006; Sica et al. 2006, 2008). Macrophage phenotypes have been shown to express different receptors and produce different cytokines, and TAMs represent a subset of alternatively activated (M2) macrophages induced by Th_2 cytokines such as IL-4 and IL-13 (Gordon 2003; Joyce and Pollard 2009; Lewis and Pollard 2006; Mosser 2003; Mantovani et al. 2004; Sica et al. 2006, 2008). Therefore, M2 macrophages in TAMs accelerate tumor growth, invasion, and metastasis. However, the antitumor and antimetastatic actions of xanthoangelol and 4-hydroxyderricin on the role of M2 macrophages in the TAMs of highly metastatic osteosarcoma LM8-bearing mice have not yet been fully clarified. In the present study, we examined the effects of xanthoangelol and 4-hydroxyderricin on tumor growth and metastasis in osteosarcoma LM-8-bearing mice (*in vivo*), and on the differentiation and activation of M2 macrophages (*in vitro*).

Materials and methods

Materials

Xanthoangelol and 4-hydroxyderricin (Baba et al. 1990; Kozawa et al., 1977, 1978) were supplied by Professor K. Baba (Department of Pharmacognosy, Osaka University of Pharmaceutical Sciences, Osaka, Japan). The purity of xanthoangelol and 4-hydroxyderricin was assessed by high performance liquid chromatography (HPLC) (Hitachi HPLC System, Hitachi, Tokyo, Japan) under the following two conditions: 1) The elution was performed with 56% acetonitrile containing 1% acetic acid (mobile phase) with a flow rate of 0.8 ml/min, monitoring wavelength at 330 nm, and Inertsil ODS-3 column (100 × 4.0 mm I.D.) (GL Science. Tokyo, Japan) (Fig. 2a); 2) The elution was performed with 70% methanol containing 1% acetic acid (mobile phase) with a flow rate of 0.75 ml/min, monitoring wavelength at 330 nm, and Inertsil ODS-3 column (100 × 4.0 mm I.D.) (Fig. 2b). The purity of xanthangelol and 4-hydroxyderricin was over 99.1% by the HPLC analysis using the above solvents (Fig. 2). Dulbecco's Modified Eagle's Medium (DMEM) and RPMI-1640 medium were obtained from Nissui Pharmacy Co. (Tokyo, Japan). Antibiotic and antimycotic solutions (100×) containing 10,000 units of penicillin, 10 mg/ml streptomycin, and 25 μg/ml of amphotericin B in 0.9% NaCl solution were purchased from Sigma–Aldrich (Tokyo, Japan). Fetal bovine serum (FBS) was purchased from Gibco BRL (Auckland, New Zealand). One-hundred-millimeter culture dishes were purchased from Corning Glass Works (NY, USA). The human interleukin (IL)-10 and monocyte chemoattractant protein (MCP)-enzyme-linked immunosorbent assay (ELISA) kits were purchased from R & D Systems Inc. (MN, USA). Human recombinant IL-3 and IL-4 were purchased from R & D Systems Inc. The rabbit monoclonal anti-Stat 3 and rabbit monoclonal anti-phospho Stat 3 (Tyr 705) antibodies were purchased from Cell Signaling Technology Inc. (MA, USA). The mouse monoclonal anti-β-action antibody was purchased from Sigma–Aldrich. The anti-F4/80 antibody was purchased from AbD Serotec (NC, USA). γ-Cyclodextrin was purchased from Ensuiko Sugar Refinding Co. Ltd. (Yokohama, Japan). Cisplatin (CDDP) was supplied by Nihon Kayaku Co. (Tokyo, Japan) and dissolved in 0.9% NaCl. All chemicals used in this study were of

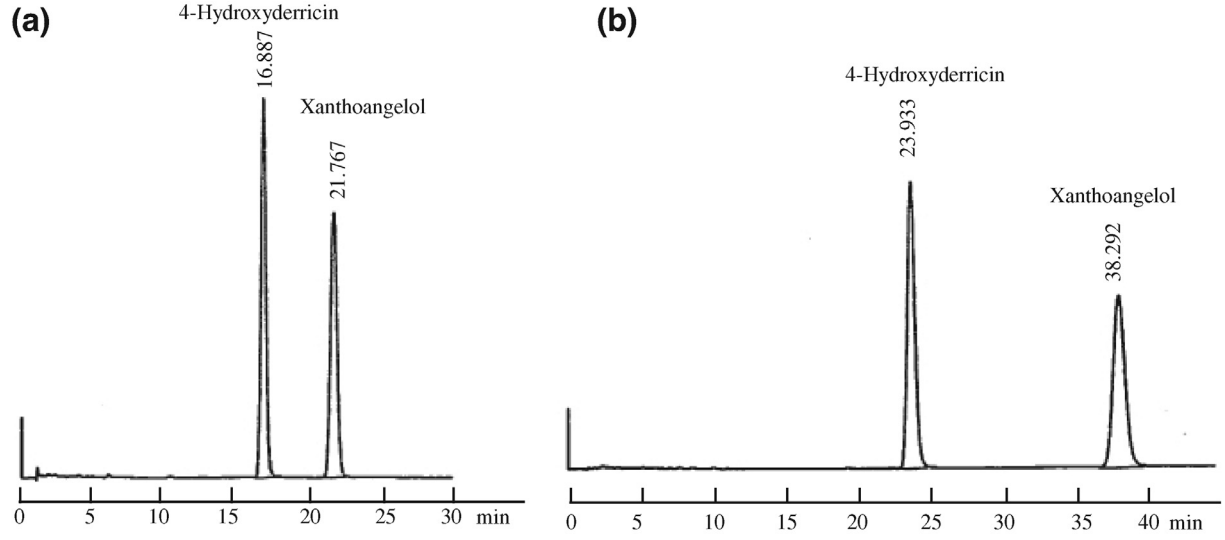

Fig. 2. HPLC profiles of xanthoangelol and 4-hydroxyderricin performed in a Inertsil ODS-3 column (100 × 4.0 mm I.D.) in the following two elution solvent systems. (a) 56% acetonitrile containing 1% acetic acid; flow rate: 0.8 ml/min, monitored at 330 nm. (b) 70% methanol containing 1% acetic acid; flow rate: 0.75 ml/min, monitored at 330 nm.