Available online at SciVarse ScienceDirect
Chinese Herbal Medicines (CHM)
ISSN 1674-6384
Journal homepage: www.tiprpress.com E-mail: chm@tiprpress.com

Original article

Inhibition of Ovalitenin A on Proliferation of HeLa Cells via Apoptosis, G_2/M Cell Cycle Arrest, and Down-regulation of COX-2

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ARTICLE INFO

Article history

Received: January 19, 2016

Revised: March 1, 2016

Accepted: March 30, 2016

Available online: July 6, 2016

DOI:

10.1016/S1674-6384(16)60048-5

ABSTRACT

Objective Ovalitenin A (1-(4-methoxybenzofuran-5-yl)-3-phenyl-2-propen-1-one) is a chalcone isolated from *Millettia pulchra*. The aim of the study was to investigate the antitumor effect of ovalitenin A on apoptosis *in vitro* and *in vivo* and to identify the mechanism involved. **Methods** The effect of ovalitenin A in human cervical cancer HeLa cells was detected by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, morphological observation, flow cytometric measurement, Western blotting, and xenograft model. **Results** Ovalitenin A inhibited the proliferation of HeLa cells in a dose-dependent manner *in vitro* and *in vivo* and induced the apoptosis evidenced by characteristic apoptotic morphological changes, phosphatidylserine externalization, and activation of caspase-3. In addition, ovalitenin A induced G_2/M cell cycle arrest and up-regulation of the Bax/Bcl-2 ratio. Furthermore, ovalitenin A decreased protein level of COX-2 and induced the loss of mitochondrial membrane potential. **Conclusion** These data suggest that ovalitenin A has the potential of anticancer properties for the treatment of cervical cancer.

Key words

apoptosis; COX-2; HeLa cell; ovalitenin A

1. Introduction

In recent years, there has been a growing interest in the use of herbs as a potent source of new therapeutic anticancer drugs. Some plant chalcones, which could induce cell cycle arrest and apoptosis in different cancer cell lines, have been identified as chemopreventive or anticancer agents (Yit and Das, 1994; Anto et al, 1995; Ducki et al, 1998). Butein, the most potent chalcone, exhibited the cytotoxic effect on the proliferation of human colon adenocarcinoma cells and induced G_2/M cell phase arrest and apoptosis in human hepatoma cancer cells through ROS generation (Yit and Das, 1994; Moon et al, 2010). Isoliquiritigenin, a simple chalcone derivative, induced the apoptosis of gastric and prostate cancer cells, which was mediated through mitochondrial events, including disruption of mitochondrial membrane potential, the release of cytochrome *c* and Smac/Diablo, and the activation of caspase-9 (Jung et al, 2006; Ma et al, 2001).

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Fund: Comprehensive Technology Platform of New Drug Research of the Ministry of Science and Technology (2012ZX09301-002-001).

Phloretin, a major apple chalcone, induced the apoptosis of B16 melanoma cells, which was suggested to result from the inhibition of glucose transmembrane transport, inhibition of lipoxygenase activity, promotion of Bax protein expression, or activation of caspases (Kobori et al, 1997; 1999). E-2-(4′-methoxybenzylidene)-1-benzosuberone, a synthetic chalcone analogue, decreased the secretion of matrix metalloproteinase (mainly MMP-9) and vascular endothelial growth factor (VEGF) (Pilatova et al, 2010).

The genus *Millettia* Wight et Arn. belongs to the family Leguminosae comprising about 200 species, most of which are found in Asia, Africa, and Australia. Phytochemical research revealed that flavones and isoflavones with annellated furan and pyran rings are the main constituents, which are found to show biological activities against several kinds of cancer cells (Ito et al, 2004; 2006; Mai et al, 2010). *Millettia pulchra* Kurz var. *laxior* (Dunn) Z. Wei, which has been used traditionally in China for anti-inflammatory, immunomodulatory, and anti-fatigue effects, is widely distributed in the southwest of China. The extract of *M. pulchra* showed anti-inflammatory effect and decreased blood pressure in normal and spontaneously hypertensive rats significantly (Huang et al, 2006; 2008). The antitumor effect of the components in *M. pulchra* has not been reported. In the present study, we isolated ovalitenin A [1-(4-methoxy-benzofuran-5-yl)-3-phenyl-2-propen-1-one, Figure 1A], a chalcone from the dry root tuber of *M. pulchra*, and investigated its antiproliferative activity. The results showed that ovalitenin A could induce the apoptosis of HeLa cells and activate the mitochondrial pathway, reduce COX-2 and PARP-1 expression and Bcl-2-Bax ratio.

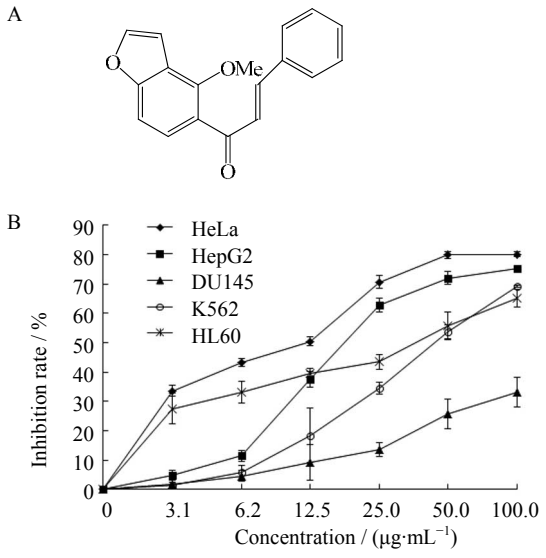

Figure 1 Chemical structure of ovalitenin A (A) and effect of ovalitenin A on tumor cell proliferation (B)

2. Materials and methods

2.1 Chemicals and reagents

RPMI-1640 medium, DMEM medium, penicillin, streptomycin, and all other tissue culture reagents were obtained from Gibco/BRL Life Technologies (USA). Fetal bovine serum (FBS) was purchased from HyClone Laboratories (USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), Hoechst 33258, ribonuclease (RNase), and propidium iodide (PI) were purchased from Sigma Chemical (USA). JC-1 was purchased from BioVision (USA), other chemicals were of analytical grade and from commercial suppliers. Primary antibodies for β-actin, NF-κB p65, Bax, Bcl-2, poly (ADP-ribose) polymerase (PARP), and COX-2 (goat polyclonal antibody) and the peroxidase-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology (USA). Paclitaxel was obtained from Beijing Xiehe Medicine Co., Ltd. (China).

2.2 Cell lines and cell culture

Five human cancer cell lines including human prostate cancer cells DU145, human cervical cancer cells HeLa, human chronic myelogenous leukemia K562, human hepatoma cells HepG2, and human leukemia HL60 were obtained from Cell Bank of Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (China). Cells were cultured in DMEM or RPMI-1640 medium supplemented with 10% heat-inactivated FBS, 1% glutamine, 100 μg/mL streptomycin, and 100 U/mL penicillin. Cells were grown in a humidified incubator at 37 °C under 5% CO_2 and used for assays during exponential phase of growth.

2.3 Preparation of ovalitenin A

The stems of *M. pulchra* were collected from Guangxi province, China, in June 2005 and taxonomically identified by Prof. Yu-lin Lin, Institute of Medicinal Plant Development, Chinese Academy of Medical Science and Peking Union Medical College. A voucher specimen (No. ALH-04-0618) was also deposited there for future reference. The air-dried stems were powdered and extracted with 90% aqueous ethanol for three times (1 h each time) under reflux. The ethanol extract was evaporated under reduced pressure to yield a syrup-like residue, which was mixed with siliceous earth and eluted with hexane, ethyl acetate, and methanol to give three fractions. A portion of the hexane extract was subjected to silica gel column chromatography (Φ7 × 90 cm) with a gradient elution of hexane-acetone (100:1, 50:1, 20:1, 10:1, 5:1, and 2:1, 5 L each eluent) to give six fractions (Frs. A–F). Fr. A was applied repeatedly to column chromatography over silica gel (Φ3 × 50 cm, hexane-acetone, 50:1) and then Sephadex LH-20 (Φ1.7 × 80 cm, chloroform-methanol, 1:1) to afford the compound, which was identified as ovalitenin A by comparison spectrum data with literature (Lee and Morehead, 1995).

2.4 Cytotoxicity against tumor cell lines

The cytotoxic potential of ovalitenin A was evaluated by