Food and Chemical Toxicology 55 (2013) 214–221

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Jaceosidin, isolated from dietary mugwort (*Artemisia princeps*), induces G2/M cell cycle arrest by inactivating cdc25C-cdc2 via ATM-Chk1/2 activation

Jong-Gyu Lee [a,b], Ji-Hyun Kim [a,b], Ji-Hye Ahn [a,b], Kyung-Tae Lee [a], Nam-In Baek [c], Jung-Hye Choi [a,b,*]

[a] Department of Life & Nanopharmaceutical Science, Kyung Hee University, Seoul, South Korea
[b] Department of Oriental Pharmaceutical Science, Kyung Hee University, Seoul, South Korea
[c] Graduate School of Biotechnology and Plant Metabolism Research Center, Kyung Hee University, Suwon, South Korea

ARTICLE INFO

Article history:
Received 26 July 2012
Accepted 18 December 2012
Available online 26 December 2012

Keywords:
Mugwort
Jaceosidin
Endometrial cancer
ATM
Cdc25C
Chk1/2

ABSTRACT

Jaceosidin, a flavonoid derived from *Artemisia princeps* (Japanese mugwort), has been shown to inhibit the growth of several human cancer cells, However, the exact mechanism for the cytotoxic effect of jaceosidin is not completely understood. In this study, we investigated the molecular mechanism involved in the antiproliferative effect of jaceosidin in human endometrial cancer cells. We demonstrated that jaceosidin is a more potent inhibitor of cell growth than cisplatin in human endometrial cancer cells. In contrast, jaceosidin-induced cytotoxicity in normal endometrial cells was lower than that observed for cisplatin. Jaceosidin induced G2/M phase cell cycle arrest and modulated the levels of cyclin B and p-Cdc2 in Hec1A cells. Knockdown of p21 using specific siRNAs partially abrogated jaceosidin-induced cell growth inhibition. Additional mechanistic studies revealed that jaceosidin treatment resulted in an increase in phosphorylation of Cdc25C and ATM-Chk1/2. Ku55933, an ATM inhibitor, reversed jaceosidin-induced cell growth inhibition, in part. Moreover, jaceosidin treatment resulted in phosphorylation of ERK, and pretreatment with the ERK inhibitor, PD98059, attenuated cell growth inhibition by jaceosidin. These data suggest that jaceosidin, isolated from Japanese mugwort, modulates the ERK/ATM/Chk1/2 pathway, leading to inactivation of the Cdc2-cyclin B1 complex, followed by G2/M cell cycle arrest in endometrial cancer cells.

1. Introduction

Artemisia princeps (Japanese mugwort) is a familiar plant that is widely used in East Asia as a food substance and medicinal herb. In Japan and Korea, it is called yomogi and ssuk, respectively, and its leaves are commonly used for teas, spices, and cooking ingredient. The main constituents of *A. princeps* are eupatilin and jaceosidin (Fig. 1A), which are part of the flavonoid family. *A. princeps* extract and its flavonoid components have been shown to have anti-hyperglycaemic (Choi et al., 2011), lipid lowering (Jung et al., 2009; Yamamoto et al., 2011), anti-oxidative (Choi et al., 2008; Nugroho et al., 2010), and anti-inflammatory (Chang et al., 2009; Min et al., 2009) properties. Additionally, they have been reported to have anti-cancer effects against several cancers (Cho et al., 2011; Ju et al., 2012; Park et al., 2008; Sarath et al., 2007). Only a few studies on jaceosidin have been reported while there are many reports on the bioactive properties of eupatilin, and the molecular mechanisms underlying jaceosidin's anti-cancer activity are poorly understood. Therefore, in the present study, we investigated the anti-cancer effect of dietary flavonoid jaceosidin isolated from *A. princeps* and its molecular mechanism of action in human endometrial cancer cells.

Endometrial cancer is the most prevalent gynaecological malignancy. Although most women with endometrial cancer have a relatively good prognosis (Jemal et al., 2009), therapeutic options for advanced and recurrent endometrial cancer remain limited. The survival rates for patients with terminal stage and recurrent endometrial cancer are only 18% and 7.7%, respectively (Creasman et al., 2001). Thus, there is an urgent need for new preventive and therapeutic agents for late-stage and recurrent endometrial cancer.

Cell cycle de-regulation resulting in uncontrolled cell proliferation is one of the most frequent alterations that occurs during tumor development (Collins et al., 1997). Therefore, cell cycle blockade is regarded as an effective strategy for eliminating cancer

Abbreviations: ATM, atazia-telagiectasia mutated; Caspase, cystein aspartyl-specific protease; Cdc 2, cell division cycle 2; Cdk, cyclin-dependent kinase; CDKI, cyclin-dependent kinase inhibitor; ERK, extracellular signal-regulated kinase; MAPK, mitogen activated protein kinase; MTT, 3[4-dimethylthiazol-2-71]-2-5-diphenyl tetrazolium bromide; PI, propidium iodide; PI3K, phosphatidylinositol 3-kinase; siRNA, small interfering RNA.

* Corresponding author at: Department of Oriental Pharmaceutical Science, College of Pharmacy, Kyung-Hee University, Dongdaemun-Gu, Hoegi-Dong, Seoul 130-701, South Korea. Tel.: +82 2 961 0860; fax: +82 2 962 0860.
E-mail address: jchoi@khu.ac.kr (J.-H. Choi).

0278-6915/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fct.2012.12.026

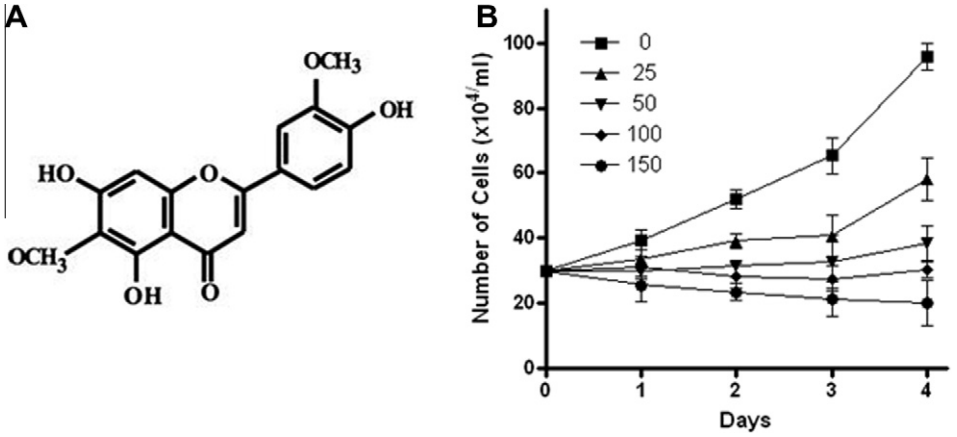

Fig. 1. Growth inhibitory effects of jaceosidin on human endometrial cancer Hec1A cells. (A) Chemical structure of jaceosidin. (B) Exponentially growing cells were treated with the indicated concentration of jaceosidin for 4 days (■, control; ▲, 25 μM; ▼, 50 μM; ♦, 100 μM; ●, 150 μM). Inhibition of cell growth was assessed by the trypan blue exclusion test, as described in method. The data shown represent the mean ± SD of three independent experiments. $^{*}p < 0.05$ vs. the control group.

cells (Buolamwini, 2000; Hajduch et al., 1999). Among the major regulated cell cycle checkpoints, the G2/M checkpoint is known to maintain chromosomal integrity by allowing cells to repair DNA damage before entering mitosis. In response to DNA damage, molecular sensors such as ataxia telangiectasia mutated (ATM) can be activated, which initiate signal transduction pathways that lead to cell cycle arrest and allow time to correct the damage (Sherr, 2000). ATM phosphorylates Chk2 on threonine 68 (Thr-68) and Chk1 on serine 317 and 345 (Ser-317 and Ser-345), resulting in their activation (Matsuoka et al., 1998; Melchionna et al., 2000). Phosphorylation of Cdc25C, which is controlled by Chk1 and Chk2 activation, is involved in the G2/M transition (Bartek and Lukas, 2003). At the onset of mitosis, Cdc2 cyclin-dependent kinase and Cyclin B complexes are activated by Cdc25C-mediated dephosphorylation of the inhibitory sites on Cdc2 (Nurse, 1990). Cyclin B-Cdc2 complexes are bound by $p21^{CIP1/WAF1}$, a member of the cyclin-dependent kinase inhibitor (CDKI) family, rendering the complex inactive (Ando et al., 2001; Harper et al., 1993).

2. Materials and methods

2.1. Materials

Jaceosidin (Fig. 1A) used for this study was isolated from *Artemisia princeps Pampanini* (AP) as described previously (Min et al., 2009). Briefly, AP cultivated in the field of GangHwa-Do, Korea, was collected and deposited in the Laboratory of Natural Product Chemistry, Kyung Hee University, Korea, with a voucher specimen (KHU05067). AP was extracted with 80% ethanol, evaporated under reduced pressure, suspended in water, and extracted, stepwise, with ethyl acetate (EtOAc). The EtOAc fraction (47 g) was chromatographed on silica gel (4 cm × 20 cm) with a step gradient of *n*-hexane–EtOAc (7:1, 5:1, 3:1, 1:1, v/v) to give 20 fractions (SSE-1–SSE-20) at the first column. The SSE-16 fraction (1.53 g) was re-separated on silica gel (4 cm × 20 cm) with $CHCl_3$–MeOH solvent pairs (30:1, v/v) to obtain jaceosidin (SSE-16-4, 127 mg). Jaceosidin (purity, >95%) was identified based on physicochemical properties and spectroscopic analysis and by comparison with the literature values. The purity was analyzed using a HPLC system (Yonunglin Instrument, Korea).

RPMI 1640, fetal bovine serum (FBS), penicillin, and streptomycin were obtained from Life Technologies Inc. (Grand Island, NY, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) was purchased from Molecular Probes Inc. (Eugene, OR, USA). Propidium iodide (PI) was purchased from Sigma Chemical (St. Louis, MO, USA). Phenylmethylsulfonylfluoride (PMSF) was purchased from BD Biosciences (San Jose, CA, USA). Antibodies for cyclin B1, phospho-Cdc2 (Tyr 15), Cdc2, phospho-ATM, p21, p27, caspase-3, and β-actin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and phospho-Cdc25C (Ser 216), Cdc25C, phospho-Akt, total-Akt, phospho-ERK1/2, total-ERK1/2, phospho-H2AX, H2AX and phospho-Chk2 from Cell Signaling (Beverly, MA, USA). We also pre-treated the cells with 10 μM of PD98059 (Invitrogen, Carlsbad, CA) and 3 μM of Ku55933 (Selleck, USA) for 60 min in inhibitor study.

2.2. Trypan blue assay

The *in vitro* growth inhibitory effect of jaceosidin on the Hec1A cells was determined by trypan blue dye exclusion. The reduction in viable cell number was assessed for each 4 days. The cells were seeded at a concentration of 3×10^5 cells/ml and were maintained for logarithmic growth by passaging them every 2–4 days, and incubated for 1–4 days with jaceosidin at various concentrations. Jaceosidin dissolved in DMSO was added to the medium in serial dilution (the final DMSO concentration in all assays did not exceeded 0.1%). Cells were loaded on a hemocytometer, and viable cell number was determined based on exclusion of trypan blue dye.

2.3. Cell culture and MTT assay

The endometrial cancer cell lines Hec1A and KLE are originally from American type culture collection. The normal endometrial cells HES, recently established by Dr. Krikun (Yale University, New Haven, Connecticut, USA), and HESC were kindly provided by Dr. Asgi Fazleabas of University of Illinois at Chicago (USA). Cells were cultured in DMEM supplemented with 5% fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin sulfate (100 μg/mL). The cytotoxicity was assessed using a MTT assay. Briefly, the cells (5×10^4) were seeded in each well containing 50 μL of DMEM medium in a 96-well plate. After 24 h, various concentrations of jaceosidin (3.125, 6.25, 12.5, 25, 50, and 100 μM) or cisplatin (12.5, 25, 50, 100, 200, and 250 μM) were added. After 48 h, 25 μL of MTT (5 mg/mL stock solution) was added, and the plates were incubated for an additional 4 h. The medium was discarded, and the formazan blue, which was formed in the cells, was dissolved in 50 μL DMSO. The optical density was measured at 540 nm using a microplate spectrophotometer (SpectraMax; Molecular Devices, Sunnyvale, CA, USA).

2.4. Propidium iodide (PI) staining for cell cycle analysis

On the day of collection, the cells were harvested and washed twice with ice-cold PBS. The cells were fixed and permeabilised with 70% ice-cold ethanol at 4 °C for 1 h. The cells were washed once with PBS and resuspended in a staining solution containing propidium iodide (50 μL/ml) and RNase A (250 μg/ml). The cell suspensions were incubated for 30 min at room temperature followed by fluorescence-activated cell sorting (FACS) cater-plus flow cytometry (Becton Dickinson Co., Germany) using 10,000 cells per each group.

2.5. Western blot analysis

Jaceosidin-treated cells were washed with ice-cold PBS and extracted in protein lysis buffer (Intron, South Korea). Protein concentration was determined by a Bradford assay. Protein samples of cell lysate were mixed with an equal volume of 5× SDS sample buffer, boiled for 4 min, and then separated on 10–12% SDS–PAGE gels. After electrophoresis, proteins were transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were blocked in 5% non-fat dry milk for 1 h, washed, and incubated with specific antibodies (cyclin B1, p21, p27, β-actin, phospho-Cdc2 (Tyr 15), Cdc2, phospho-Cdc25C (Ser 216), Cdc25C, phospho-ATM, phospho-Chk1, phospho-Chk2, phospho-Akt, total-Akt, phospho-ERK1/2, total-ERK1/2, phospho-JNK, total-JNK, phospho-p38, total-p38, phospho-H2AX, H2AX) in Tris-buffered saline (TBS) containing Tween-20 (0.1%) overnight at 4 °C. Primary antibodies were removed by washing the membranes three times in TBS-T, and then the membranes were incubated for 1 h with horseradish peroxidase-conjugated secondary antibody