Phytomedicine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Phytomedicine

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

Sinapine reverses multi-drug resistance in MCF-7/dox cancer cells by downregulating FGFR4/FRS2α-ERK1/2 pathway-mediated NF-κB activation

Q1 Ying Guo[a], Yuanyuan Ding[b], Tao Zhang[b], Hongli An[c,*]

[a] National-local Joint Engineering Research Center of Biodiagnostics & Biotherapy, Second Affiliated Hospital, School of Medicine, Xi'an Jiaotong University, Xi'an 710004, China
[b] School of Pharmacy, Xi'an Jiaotong University, Xi'an 710061, China
[c] Center for Translational Medicine, The First Affiliated Hospital of Medical College, Xi'an Jiaotong University, Xi'an 710061, China

ARTICLE INFO

Article history:
Received 25 June 2015
Revised 10 December 2015
Accepted 28 December 2015
Available online xxx

Keywords:
Sinapine
Doxorubicin
MCF-7/dox
P-glycoprotein
NF-κB
FGFR4

ABSTRACT

Sinapine, an alkaloid derived from seeds of the cruciferous species, shows favorable biological properties, such as antioxidant and radio-protective activities. The inhibitory effect of sinapine on acquired chemoresistance in tumor cells and the underlying molecular mechanisms remain unknown.

We examined the effect of sinapine on reversal of chemoresistance in Michigan Cancer Foundation 7 (MCF-7)/dox breast cancer cells.

Combination treatment with sinapine and doxorubicin synergistically increased the cytotoxicity of doxorubicin in MCF-7/dox cells, as shown using a cell apoptosis assay. An accumulation assay demonstrated that sinapine increased the intracellular concentration of doxorubicin in a dose-dependent manner. Immunoblotting and real time polymerase chain reaction (RT-PCR) analysis showed that sinapine downregulated multi-drug resistance 1 (MDR1) expression. A significant correlation was observed between the expression of MDR1, phospho-factor receptor substrate (FRS), phospho-extracellular signal regulated kinase (ERK)1/2, and nuclear factor kappa B (NF-κB). Chromatin immunoprecipitation (ChIP) assay indicated that sinapine inhibited binding of the transcription factor NF-κB to the MDR1 promoter.

Our findings indicated that sinapine played an important role in the downregulation of MDR1 expression through suppression of fibroblast growth factor receptor (FGFR)4/FRS2α-ERK1/2 mediated NF-κB activation in MCF-7/dox cancer cells.

Introduction

Drug resistance is a significant factor that limits the effectiveness of chemotherapeutic drugs. Tumors may be intrinsically resistant to chemotherapy prior to treatment, or drug resistance can be induced by the chemotherapeutic drug during treatment. Thus, tumors that are initially sensitive will frequently become resistant to chemotherapy (Longley and Johnston 2005). Several tumor drug resistance mechanisms have been described – such as p53 activation, deletion or inactivation of the pro-apoptotic gene caspase-3, increase in BCL-2 protocarcinogenic gene expression, blockade of apoptosis pathway, increased protein expression of ATP transport box, etc., – which promote resistance of tumor cells to chemotherapeutic drugs (Hipfner et al. 1999; Ozvegy et al. 2001; Roy et al. 2007; Sparrebppm et al. 2003; Sanchez et al. 2009).

Resistance of cancer cells to structurally diverse and mechanically unrelated anticancer drugs, a phenomenon termed multidrug resistance (MDR), is a major obstacle to successful cancer chemotherapy. Overexpression of P-glycoprotein (P-gp) is the most frequent cause of MDR. Expression of multidrug resistance-1 gene (*MDR1*) has been studied in certain cancer cells, including human breast cancer Michigan Cancer Foundation 7 (MCF-7) cells and its multidrug resistant subline MCF-7/dox (Xiang and Gao 2010). Molecularly, P-gp/MDR1 expression is regulated by the transcription factor nuclear factor kappa B (NF-κB) (Zhao and Sun 2013).

Sinapine, a small molecular alkaloid, is an extract from seeds of the cruciferous plants species. Bai Jie Zi (BJZ) is a traditional Chinese medicine (TCM) that is widely used in clinical practice in China (Liu et al. 2006; Zhang et al. 2013). Previous studies showed that sinapine has various pharmacological effects, such as anti-inflammatory (Bhinu et al. 2009), antioxidant (Dubie et al. 2013),

Abbreviations: FGFR, fibroblast growth factor receptor; FRS2α, FGFR substrate protein-2α; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; P-gp, P-glycoprotein; MDR, multidrug resistance; ChIP, Chromatin immunoprecipitation.

* Corresponding author. Tel./fax: +86 29 85324626.
E-mail address: anhongli@mail.xjtu.edu.cn (H. An).

http://dx.doi.org/10.1016/j.phymed.2015.12.017
0944-7113/

and anti-angiogenic properties (He 2008). However, the effect of sinapine on reversal of acquired chemoresistance has not yet been examined (Guo et al. 2014). In this study, we investigated the anti-tumor activity of sinapine and the reversal of doxorubicin resistance mechanism.

Materials and methods

Reagents

Sinapine (Fig. 1A) was purchased from Preferred Biotechnology Co. (Lot: 121122, Chengdu, China). Doxorubicin and 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). TNF-α was obtained from Genscript (Hangzhou, China), and FGF19 was obtained from Sino Biological (Beijing, China). The following primary antibodies were used: NF-κB p65 (chromatin immunoprecipitation (ChIP) grade, #ab7970) was obtained from Abcam (Cambridge, MA, USA). MDR1 (#sc-8313) was obtained from Santa Cruz Biotechnology (Dallas, TX, USA). phospho-p44/42 mitogen-activated protein kinase (MAPK) (phospho-extracellular signal regulated kinase (ERK)1/2, p44/42 MAPK (ERK1/2) (#9102S), and inhibitor of kappa B kinase (IKK)α/β (#2697) were from Cell Signaling (Beverley, MA, USA). Protein A/G PLUS-agarose immunoprecipitation reagent (#sc-2003) was from Santa Cruz Biotechnology. Roswell Park Memorial Institute (RPMI) 1640 medium was purchased from Gibco (Rockville, MD, USA).

Cell culture

The drug-sensitive human breast cancer cell line MCF-7 cell and its derivative multi-drug resistant variant, MCF-7/dox cells, were obtained from the Cell Bank of Shanghai, Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). They were maintained in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Hyclone, Logan, UT, USA) at 37 °C under humidified atmosphere containing 5% CO_2.

Measurement of cell viability

The MCF-7/dox cells were cultured in 96-well plates, and cell viability was determined by the MTT assay. After incubation with different doses of sinapine, doxorubicin, or both for 24 h, the medium was discarded. Cells were treated with MTT solution (500 μg/ml) for 4 h. The dark blue formazan crystals formed were solubilized with dimethyl sulfoxide, and the absorbance was measured at 490 nm in a microplate reader (Bio-rad, Carlsbad, CA, USA). Evaluation of drug combination was conducted by the Q index with the following formula (Jin 1980): $Q = E_{AB} / (E_A + E_B - E_A E_B)$. E_{AB} is the inhibitory rate of drug combination, and E_A and E_B represent the inhibitory rate of a single drug treatment.

Cell apoptosis

The MCF-7/dox cells (1×10^5 cells/ml) were seeded into 6-well plates. When cells reach 80% confluence, cells were incubated with 1.2 μM doxorubicin alone or together with sinapine at concentrations of 10, 50, 100, 150, and 200 μM for 24 h. The apoptotic cells were then washed twice and resuspended in ice-cold phosphate buffered saline (PBS) and detected using the Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) double staining method. Cells were incubated with Annexin V-FITC staining solution for 15 min away from light. Then, the cells were incubated with PI for 15 min away from light. The apoptotic cell death rate was analyzed using flow cytometry (FACSort; Becton Dickinson Co., Franklin Lakes, NJ, USA).

Cellular doxorubicin accumulation assay

The MCF-7/dox cells were seeded into 10-cm culture dishes for 24 h. Then, the cells were incubated with 1.2 μM doxorubicin alone or co-incubated in serial concentrations of sinapine (0, 10, 50, 150, and 200 μM) for 24 h. Cells were washed twice with cold PBS and then measured by flow cytometry (FACSort) at 488 nm.

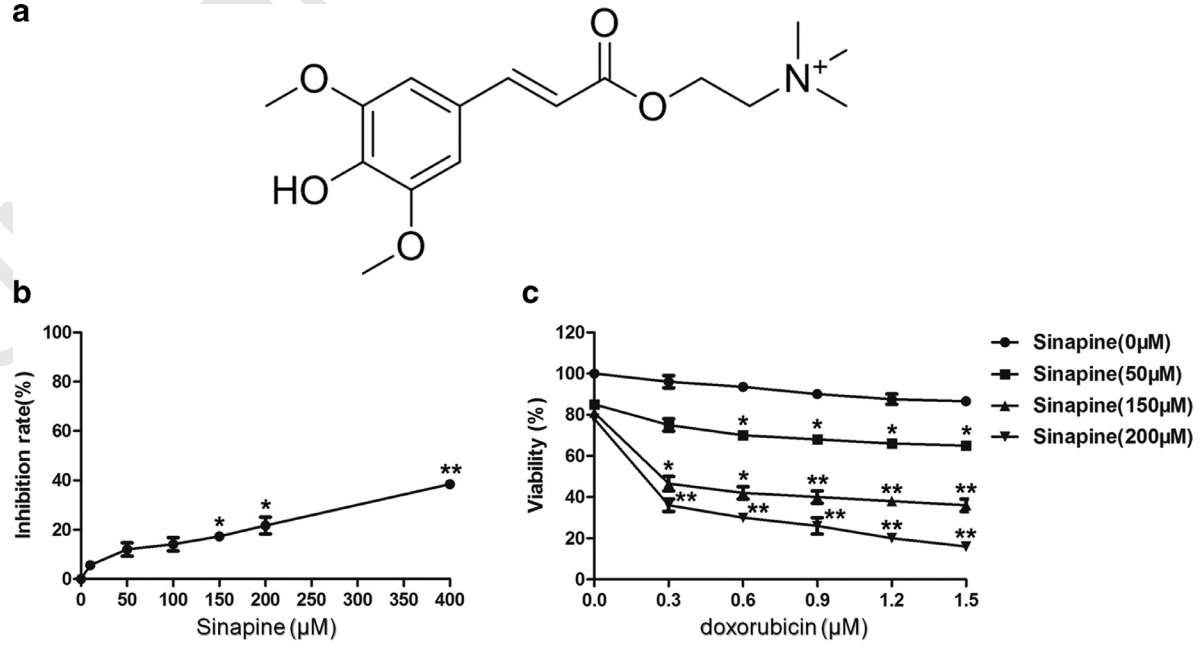

Fig. 1. Effect of sinapine on cell viability. (A) Structure of sinapine. (B) After treatment with different concentrations of sinapine in MCF-7/dox cells for 24 h, cell growth inhibition rate was assayed by MTT assay. (C) After treating with 0, 0.3, 0.6, 0.9, 1.2, or 1.5 μM doxorubicin together with 0, 50, 150, or 200 μM sinapine respectively for 24 h, the viability of MCF-7/dox cells was assayed by MTT assay. Data are presented as mean ± SD, $n = 3$. $^*p < 0.05$ vs. control, $^{**}p < 0.01$ vs. control.

Please cite this article as: Y. Guo et al., Sinapine reverses multi-drug resistance in MCF-7/dox cancer cells by downregulating FGFR4/FRS2α-ERK1/2 pathway-mediated NF-κB activation, Phytomedicine (2016), http://dx.doi.org/10.1016/j.phymed.2015.12.017