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The mechanism of ellipticine-induced apoptosis and cell cycle arrest in human breast MCF-7 cancer cells

Po-Lin Kuo[a], Ya-Ling Hsu[b], Cheng-Hsiung Chang[c], Chun-Ching Lin[b,*]

[a]*Department of Biotechnology, Chia-Nan University of Pharmacy and Science, Tainan, Taiwan, ROC*
[b]*Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung, Taiwan, ROC*
[c]*Department of Pharmacy, Chia-Nan University of Pharmacy and Science, Tainan, Taiwan, ROC*

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Abstract

Ellipticine, a cytotoxic plant alkaloid, is known to inhibit topoisomerase II. Here, we first report the molecular mechanism of ellipticine's apoptotic action in human breast MCF-7 cancer cells. Treatment of cells with ellipticine resulted in inhibition of growth, and G2/M phase arrest of the cell cycle. This effect was associated with a marked increase in the protein expression of p53 and, p21/WAF1 and KIP1/p27, but not of WAF1/p21. Ellipticine treatment increased the expression of Fas/APO-1 and its ligands, mFas ligand and sFas ligand, and subsequent activation of caspase-8. The mitochondrial apoptotic pathway amplified the Fas/Fas ligand death receptor pathway by Bid interaction. This effect was found to result in a significant increase in activation of caspase-9. Taken together, we have concluded that the molecular mechanisms during ellipticine-mediated growth inhibition and induction of apoptosis in MCF-7 cells were due to (1) cell cycle arrest and induction of apoptosis, (2) induction of p53 and KIP1/p27 expression, (3) triggering of Fas/Fas ligand pathway, (4) disruption of mitochondrial function, and (5) the apoptotic signaling was amplified by cross-talk between Fas death receptor and mitochondrial apoptotic pathway.
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Keywords: Ellipticine; Apoptosis; Fas/Fas ligand; Mitochondria

1. Introduction

Ellipticine (5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole) is one of the simplest naturally occurring alkaloids, having a planar structure [1]. It was first isolated in 1959 from the leaves of the evergreen tree *Ochrosia elliptica* Labill (Apocynaceae), which grows wild in Oceania [1]. The anticancer activity of ellipticine and its derivatives, such as 9-methoxyellipticine, retelliptine, ellipticiniums, have been reported as being selectively active against cancer cells in in vitro and in vivo studies [2–7]. Studies on the mechanisms of the cytotoxicity and anticancer activity of ellipticine and its analogs have shown that these activities to be due to (1) DNA intercalation, (2) inhibition of topoisomerase II, (3) covalent

* Corresponding author. Address: Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, No. 100, Shin-Chuan 1st Road, Kaohsiung 807, Taiwan, ROC. Tel.: +886 7 312 1101x2122; fax: +886 7 313 5215.
E-mail address: aalin@ms24.hinet.net (C.-C. Lin).

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alkylation of macromolecules, and (4) induction of endoplasmic reticulum stress [2–9].

Apoptosis signaling converges at the activation of initiator caspases (i.e. caspase-8 and caspase-9), which leads to the proteolytic activation of effectors caspase (i.e. caspase-3) that then cleaves the cellular substrate, resulting in cell death [10,11]. The death receptor pathway is triggered by members of the death receptor superfamily, such as Fas/APO-1. Ligation of Fas/APO-1 by agonistic antibody or its mature ligand (Fas ligand) induces receptor oligomerization and formation of death-inducing signaling complex (DISC), followed by activation of caspase-8. The mitochondrial apoptotic pathway is controlled by Bcl-2 family protein, including the proapoptotic Bax and antiapoptotic Bcl-2 and Bcl-X_L [12,13]. Death stimuli induce the release of cytochrome *c*, procaspase-9 and other proapoptotic factors from the mitochondria into the cytoplasm, thereby activating downstream effector caspases such as caspase-3. Cross-talk between death receptor and mitochondrial pathway is provided by Bid (a member of Bcl-2 family). Caspase-8-mediated cleavage of Bid greatly increases its pro-death activity and results in its translocation to the mitochondria, where it promotes cytochrome *c* exit [14].

In this study, to establish the anticancer mechanism of ellipticine in MCF-7 cells, we assayed the death receptor and mitochondrial apoptotic pathway-related molecules, including Fas/APO-1, Fas ligand, caspase-8, cytochrome *c*, caspase-9, and Bcl-2 family proteins, which are strongly associated with the signal transduction pathway of apoptosis and affect the chemosensitivity of tumor cells to anticancer agents.

2. Materials and methods

2.1. Materials

Fetal calf serum (FCS), penicillin G, streptomycin, amphotericin B, Dulbecco's modified Eagle's medium (DMEM) and insulin were obtained from GIBCO BRL (Gaithersburg, MD). Ellipticine, dimethyl sulfoxide (DMSO), ribonuclease (RNase), and propidium iodide (PI) were purchased from Sigma Chemical Co. (St Louis, MO). XTT and p53 pan ELISA kits were obtained from Roche Diagnostics GmbH (Germany). Nucleosome ELISA, WAF1 ELISA, Fas Ligand, Fas/APO-1 ELISA, and caspase-8, caspase-9 activity assay kits, caspase-8 inhibitor (Z-IETD-FMK) and caspase-9 inhibitor (LEDH-CHO) were purchased from Calbiochem (Cambridge, MA). The antibodies to KIP/p27, Bax, Bcl-2, and Bcl-X_L were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The antibodies to Bid and cytochrome *c* were obtained from Cell Signaling Technology (Beverly, MA).

2.2. Cell line and culture

MCF-7 (American Type Culture Collection [ATCC] HB8065) was maintained in a monolayer culture at 37 °C and 5% CO_2 in DMEM supplemented with 10% FCS, 10 U/mL of penicillin, 10 μg/mL of streptomycin, and 0.25 μg/mL of amphotericin B and 5 μg/mL of insulin.

2.3. Cell proliferation assay

Inhibition of cell proliferation by ellipticine was measured by XTT (sodium 3′-[1-(phenylamino-carbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro)benzene-sulfonic acid hydrate) assay. Briefly, cells were plated in 96-well culture plates (1×10^4 cells/well). After 24 h incubation, the cells were treated with ellipticine (0.5, 1, 2, and 3 μM) for 48 h. Fifty microliters of XTT test solution, which was prepared by mixing 5 mL of XTT-labeling reagent with 100 μL of electron coupling reagent, was then added to each well. After 4 h incubation, the absorbance was measured on an ELISA reader (Multiskan EX, Labsystems) at a test wavelength of 492 nm and a reference wavelength of 690 nm.

2.4. Cell cycle analysis

To determine cell cycle distribution analysis, 5×10^5 cells were plated in 60-mm dishes and treated with ellipticine (0, 1.5, and 3 μM) for 6 h. After treatment, the cells were collected by trypsinization, fixed in 70% ethanol, washed in PBS, resuspended in 1 mL of PBS containing 1 mg/mL RNase and 50 μg/mL propidium iodide, incubated in the dark for 30 min at room temperature, and analyzed by EPICS flow cytometer. The data were analyzed using the Multicycle software (Phoenix Flow Systems, San Diego, CA).

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