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Induction of apoptosis in K562 human leukemia cells by 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone

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Abstract

2′,4′-Dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (DMC), isolated from the buds of *Cleistocalyx operculatus*, was investigated in its cytotoxicity and anti-proliferation on K562 cell line. Our results revealed that the IC_{50} was equal to 14.2 ± 0.45 μM and the EC_{50} was 3.3 ± 0.14 μM. Staining with Hoechst 33258 showed fragmentation and condensation of chromatin in the cells treated with 8 μM DMC for 48 h. Flow cytometric analysis was performed to determine hypodiploid cells. The results of flow cytometry assay indicated that the percentage of hypodiploid K562 cells was $76.15 \pm 3.22\%$ after 48 h treatment with 16.0 μM DMC. The treatment resulted in the appearance of a hypodiploid peak (A_0 region), probably due to the presence of apoptosing cells and/or apoptotic bodies with DNA content less than 2*n*. Western blot results illustrated that in the same dosage and incubation time, DMC could down-regulate the level of Bcl-2 protein and did not influence the expression of Bax protein. The resulting net effect could thus lead to a lower ratio of Bcl-2/Bax, which might be responsible for the DMC-induced apoptosis in K562 cells.

Keywords: *Cleistocalyx operculatus*; Flavonoids; K562; Bcl-2/Bax; Apoptosis

1. Introduction

Flavonoids are polyphenolic compounds that occur naturally in normal human diet and in many folk medicines, still in use. These compounds can scavenge superoxide, hydroxy and proxyradicals, breaking lipid peroxide chain reactions. They have also been shown to protect cells from X-ray damage, to block progression of cell cycle, to inhibit mutations, to block prostaglandin synthesis and to prevent multistage carcinogenesis in experimental animals [1]. Various pharmacological activities of flavonoids have been studied extensively [2–5]. Chalcones, considered as the precursor of flavonoids and isoflavonoids, are abundant in edible plants. A number of chalcones have demonstrated cytotoxic [6,7] and anti-cancer properties [8,9].

Cleistocalyx operculatus (Roxb.) Merr. et Perry (Myrtaceae), is a well-known medicinal plant whose buds are commonly used as an ingredient for tonic drinks in southern China. It was reported that the water extract of the buds of *C. operculatus* was shown to increase the contractility and decrease the frequency of contraction in an isolated rat heart perfusion system [10]. Our previous phytochemical attention to the species has led to the characterization of sterol, flavanone, chalcone and triterpene acid from its buds [11]. In this paper, we investigated the in vitro anti-tumor activity of 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (DMC), one of the compound from the buds of *C. operculatus*, in K562 cell line. In addition, we were interested in elucidating the molecular mechanisms by which DMC may induce K562 cells apoptosis.

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2. Materials and methods

2.1. Chemicals

DMC was isolated from *C. operculatus* in our lab as described by Ye et al. [11]. Previous experiments have shown that DMC purity was above 96%. The structure of the compound is shown in Fig. 1. The compound was dissolved in dimethyl sulfoxide (DMSO). The control cells were treated with the same amount of vehicle alone. The final DMSO concentration never exceeded 0.1% (v/v), in either control or treated samples. Previous experiments have shown that DMSO at this concentration does not modify the cellular activities that we are analyzing. Fetal bovine serum (FBS) and Rosewell Park Memorial Institute (RPMI) 1640 medium were purchased from Life Technologies, Inc., MA, USA.

2.2. Cell lines and culture conditions

K562 human chronic leukemia cells were purchased from Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). Cells were cultured in RPMI 1640 medium with 10% FBS, penicillin (100 U/ml) and streptomycin (100 μg/ml) in an atmosphere of 5% CO_2 in humidified air at 37 °C. In all experiments, exponentially growing cells were used.

2.3. Cytotoxycity assay

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay was performed as described by Mosman [12]. K562 cells were placed within 96-well culture plates (10^4 cells/well). The cells were treated with DMC ranging from 12.5 to 100 μM or without (vehicle control, 0.1% dimethyl sulfoxide) DMC. DMC cytotoxycity was measured after 2 days of culture using the MTT assay. Absorbance in control and drug treated wells was measured in an Automated Microplate Reader (Bio-Rad 550) at 550 nm. The cytotoxycity of DMC was expressed as IC_{50} (concentration of the DMC that induces 50% inhibition of the cell growth, which was extrapolated from linear regression analysis of experimental data).

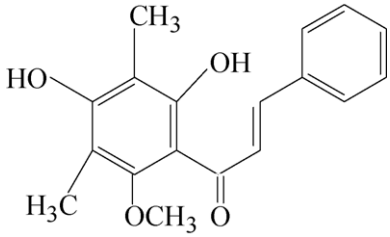

Fig. 1. Structure of 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone.

2.4. Soft agar colony forming (clonogenic) assay

The effects of DMC on soft agar colony forming of K562 cells were investigated. Briefly, K562 cells were treated with DMC ranging from 1.5 to 25 μM or without (vehicle control, 0.1% dimethyl sulfoxide) DMC and then mixed with agar in a final concentration of 0.33%. Aliquots of 1.5 ml containing 10^3 cells and 10% FBS were plated in triplicate in 35-mm culture dishes over a base layer of 0.6% agar and allowed to gel. Colonies (>20 cells) were counted after 14 days of incubation under an inverted microscope (Leica DMIRB, Germany) [13]. The anti-proliferative activity was expressed as EC_{50} (concentration of the DMC that induces 50% inhibition of the cell colony number, which was extrapolated from linear regression analysis of experimental data).

2.5. Fluorescent staining of nuclei for K562 cells

K562 cells from exponentially growing cultures were seeded within 24-well culture plates. The cells were treated with 8 μM DMC or without (vehicle control, 0.1% dimethyl sulfoxide) DMC for 48 h. After treatment, cells were washed with phosphate-buffered saline (PBS), and were fixed in MeOH–HAc (3:1, v/v) for 10 min at 4 °C. Cells were stained with Hoechst 33258 (5 μg/ml in PBS) for 5 min at room temperature and then examined in a Leica DMIRB fluorescent microscope at 356 nm [14].

2.6. Flow cytometry assay for K562 cells

Flow cytometric analysis was performed to determine hypodiploid cells. The cells were stained with propidium iodide using a cycle TEST PLUS DNA Reagent kit (Becton Dickinson, USA), then analysed by a FACScan (Becton Dickinson, USA) with Cell Fit software. Hypodiploid cells have less DNA than that of diploid cells at G_1 phase because of apoptosis-induced DNA fragmentation.

2.7. Protein extraction and Western blot analysis

K562 cells were planted and cultured in complete medium. The cells were treated with DMC ranging from 2 to 16 μM or without (vehicle control, 0.1% dimethyl sulfoxide) DMC. Cells were washed twice with cold PBS and lysed at 4 °C for 30 min in lysis buffer (0.5% Triton X-100, 300 mM NaCl, 50 mM Tris–Cl, 1 mM phenylmethylsulfonyl fluoride) with occasional vortexing. Insoluble material was removed by centrifugation at 4 °C for 15 min at 14,000 × *g* and the total proteins extracted were quantified by the coomassie brilliant blue G-250 assay with bovine serum albumin (BSA) as standard (VARIAN Cary 500 UV/Vis spectrophotometer, USA) [15]. Cellular proteins (40 μg/lane) were resolved on SDS–12% polyacrylamide gels and transferred to TotalBlot PVDF membranes (Amresco Inc., USA), which were blocked with 5% fat-free milk and immunostained with 1:1000 dilution of monoclonal mouse anti-Bcl-2 antibody (Santa Cruz