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Reversal of multidrug resistance by icaritin in doxorubicin-resistant human osteosarcoma cells

WANG Zhen-Dong[Δ], WANG Rui-Zhi[Δ], XIA Yuan-Zheng, KONG Ling-Yi[*], YANG Lei[*]

State Key Laboratory of Natural Medicines, Department of Natural Medicinal Chemistry, China Pharmaceutical University, Nanjing 210009, China

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[ABSTRACT] Multidrug resistance (MDR) is one of the major obstacles in cancer chemotherapy. Our previous study has shown that icariin could reverse MDR in MG-63 doxorubicin-resistant (MG-63/DOX) cells. It is reported that icariin is usually metabolized to icariside II and icaritin. Herein, we investigated the effects of icariin, icariside II, and icaritin (ICT) on reversing MDR in MG-63/DOX cells. Among these compounds, ICT exhibited strongest effect and showed no obvious cytotoxicity effect on both MG-63 and MG-63/DOX cells ranging from 1 to 10 μmol·L^{-1}. Furthermore, ICT increased accumulation of rhodamine 123 and 6-carboxyfluorescein diacetate and enhanced DOX-induced apoptosis in MG-63/DOX cells in a dose-dependent manner. Further studies demonstrated that ICT decreased the mRNA and protein levels of multidrug resistance protein 1 (MDR1) and multidrug resistance-associated protein 1 (MRP1). We also verified that blockade of STAT3 phosphorylation was involved in the reversal effect of multidrug resistance in MG-63/DOX cells. Taken together, these results indicated that ICT may be a potential candidate in chemotherapy for osteosarcoma.

[KEY WORDS] Osteosarcoma; Icaritin; Multidrug resistance; MDR1; MRP1

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Introduction

Multidrug resistance (MDR) is a protective mechanism of cancer cells against a variety of drugs with different structures and/or functions [1-2]. MDR is one of the major causes of failure in chemotherapy. Lots of mechanisms account for the chemotherapy resistant, including altered DNA repair, alterations in scavenging enzymes, and increased drug efflux [3]. Overexpression of P-gp (MDR1), MRP1 or BCRP in many human cancers is the most extensively studied mechanism in many human cancers [4-5]. These main transmembrane proteins of ATP-binding cassette transporters act as efflux pump, decreasing intracellular drug levels and reducing cytotoxic activity. The ongoing research focus on third-generation clinically used MDR modulators [6-8]. Although they are more potent and less toxic than first-generation modulators, but still have the problems of side effects, poor solubility, and unfavorable changes in pharmacokinetics, which limit their clinical benefits [9]. These have spurred on efforts searching for more effective compounds with minimal adverse side effects.

Agents derived from plant origin are being increasingly utilized in drug discovery and drug development programs [10-11]. Identification of natural compounds capable of circumventing MDR with minimal adverse side effects is an attractive goal. Icariin, icariside II, and icaritin are the main active flavonoids isolated from *Herba Epimedii* (HEP), which is widely used in China for the treatment of osteoporosis [12]. In our previous study, we have found that icariin could reverse the multidrug resistance induced by doxorubicin [13]. Icariin is hydrolyzed to icariside II and then to icaritin (Fig. 1A) before absorption [14], and the two hydrolytic metabolites may also be pharmacologically active. The present study was undertaken to evaluate the effects and the underlying mechanism of action for icaritin on MDR.

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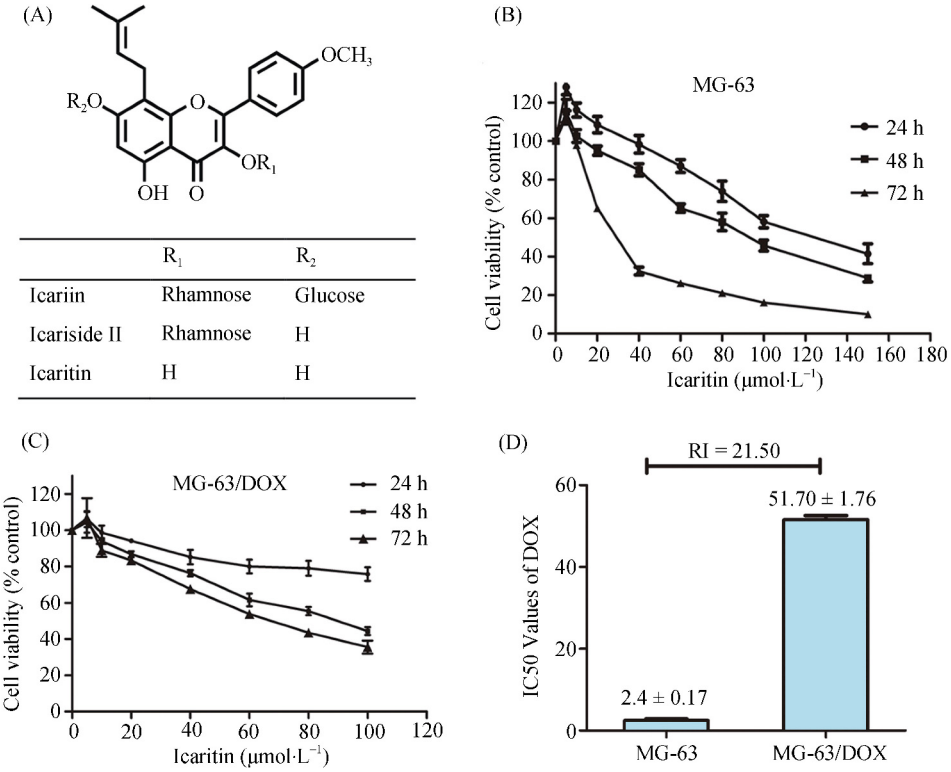
[*Corresponding author] Tel/Fax: 86-25-83271405, E-mails: cpu_lykong@ 126.com (KONG Ling-Yi); dorothy19802003@gmail.com (YANG Lei).

Δ These authors contributed equally to this work.

These authors have no conflict of interest to declare.



	R_1	R_2
Icariin	Rhamnose	Glucose
Icariside II	Rhamnose	H
Icaritin	H	H

Fig. 1 Chemical structures of the tested compounds and effects of icaritin on cell viability in MG-63 and MG-63/DOX cells. (A) the chemical structures of icariin, icariside II, and icaritin. (B) MG-63 cells were treated with different concentrations (0−150 μmol·L^{-1}) of icaritin for 24, 48, and 72 h. The cell viability was determined by MTT assay. The data are indicated as means ± SD of three independent experiments. (C) MG-63/DOX cells were treated with different concentrations (0−150 μmol·L^{-1}) of icaritin for 24, 48, and 72 h. The cell viability was determined by MTT assay. The data are represented as means ± SD of three independent experiments

In the present study, we found that ICT could more effectively reverse MDR than icariin in the DOX-resistant human osteosarcoma (MG-63/DOX) cells. It could restore the cytotoxicity of DOX in MG-63/DOX cells by down-regulation of MDR1 and MRP1. The mechanism also involved the inhibition of STAT3 phosphorylation. The results indicated that ICT might be a new drug candidate for the treatment of MDR.

Materials and Methods

Chemicals

Doxorubicin (DOX) was from Santa Cruz Biotechnology (Texas, USA). Icariin ($C_{33}H_{40}O_{15}$, MW: 676.66, purity ≥ 98%) was from J&K Scientific (Beijing, China). Verapamil (Vera), MTT, dimethyl sulfoxide (DMSO), rhodamine 123 (Rh123), sodium pyruvate, probenecid (PRB) and 6-carboxyfluorescein diacetate (CFDA) were from Sigma-Aldrich (Missouri, USA). FITC conjugated mouse anti-human monoclonal antibody against MDR1 or MRP1 were bought from Bioscience (California, USA). Annexin V-FITC kit was from Miltenyi Biotec Inc. (Auburn, USA). Antibodies against p-STAT3, STAT3, p-ERK1/2, ERK1/2, p-AKT, AKT, and GAPDH were obtained from Cell Signaling Technology. MDR1, MRP1, BCRP and horseradish peroxidase (HRP)-conjugated secondary antibody were the products of Santa Cruz Biotechnology. All other chemicals used in the present study were of reagent grade and commercially available.

Preparation and identification of icariside II and icaritin

Icariside II was prepared by *β*-glucosidase hydrolysis of icariin [15], and icaritin (ICT) was prepared by cellulase hydrolysis of icariside II [16]. The purity of the compounds was greater than 98% as determined by high performance liquid chromatography (HPLC). Stock solutions of icariin, icariside II, and icaritin were prepared in DMSO and stored at 4 °C. The final concentration of DMSO used in the cell culture was 0.1% or less.

Cell lines and cell culture

The human osteosarcoma cell line MG-63 (purchased from Typical Culture Preservation Commission Cell Bank, Shanghai, China) and its doxorubicin resistant subline MG-63/DOX established and maintained in our laboratory [13] were cultured in flasks with MEM supplemented with 10% heat-inactivated FBS, 2.2 g·L^{-1} $NaHCO_3$ and 0.11 g·L^{-1} sodium pyruvate at 37 °C in a humidified atmosphere containing 5% CO_2. The MG-63/DOX cells were incubated in the drug-free medium for 3 days before the experiments.

MTT assay

Cell growth was measured using the MTT assay. The MG-63 and MG-63/DOX cells were plated into 96-well microtiter plates at 5 × 10^3 cells per well for 24 h. For cell vi-