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Pomolic acid triggers mitochondria-dependent apoptotic cell death in leukemia cell line

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Abstract

One of the major goals in chemotherapy is to circumvent anti-apoptotic strategies developed by tumor cells. In a previous paper, we showed that pomolic acid (PA) is able to kill the leukemia cell line K562 and its MDR derivative, Lucena 1. Here, we demonstrated that PA-induced apoptosis of HL-60 cells is dependent on the activation of caspases-3 and -9 and dissipation of the mitochondrial transmembrane potential ($\Delta\psi_m$). Disruption of $\Delta\psi_m$ precedes caspase activation and is not inhibited by zVAD-fmk indicating mitochondria as the main target of PA. Our data pointed to the potential use of PA to overcome apoptosis resistance.

Keywords: Pomolic acid; Apoptosis; Mitochondria membrane potential; Cancer therapy; Leukemia; MDR

1. Introduction

One of the major causes of chemotherapeutic failures in cancer treatment is the development of different kinds of resistance. The search for new drugs able to overcome the resistance mechanisms and leading to tumor cell death is of utmost importance for cancer therapy. In a previous study [1] we showed that pomolic acid (PA), a pentacyclic triterpene isolated from *Chrysobalanus icaco*, was highly effective in inhibiting the growth of both

Abbreviations: $\Delta\psi_m$, mitochondrial transmembrane potential; ANT, adenine nucleotide translocator; Apaf-1, apoptotic protease activating factor-1; CD95-L, CD95-ligand; CsA, cyclosporin; $DiOC_6(3)$, 3,3′-dihexyloxacarbocyanide iodide; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; PI, propidium iodide; PTP, permeability transition pore; zVAD-fmk, benzyloxycarbonyl-Val-Ala-Asp(OMe)-fluoromethylketone.

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K562, a leukemia cell line and Lucena-1, a MDR resistant leukemia line that overexpresses P-glycoprotein (Pgp 170) [2]. However, apart from its capacity to induce apoptosis [1], the mechanisms of PA activity are presently unclear. As part of the evaluation of the potential of new anti-cancer compounds, this paper investigated the molecular pathways of PA-induced apoptosis.

Several compounds used in anticancer chemotherapy are believed to induce cell death via activation of key elements of the apoptotic program. Apoptosis is characterized by distinct morphological features such as plasma membrane blebbing, cell shrinkage, chromatin condensation, DNA fragmentation, and breakdown of the cell into apoptotic bodies [3–5]. Recently, it has been shown that mitochondria play an essential role in the so-called intrinsic pathway of apoptosis [6], the major route used by several chemotherapeutic drugs [7,8]. Activation of the mitochondrial permeability transition pore (PTP) leads to dissipation of mitochondria membrane potential ($\Delta\psi_m$) [9–11]. In the cytosol, the complex formed by cytochrome c, Apaf-1 and pro-caspase-9 (apoptosome) triggers the activation of caspase-9 [12]. This caspase promotes the activation of a cascade of caspases including caspase-3, with the consequent cleavage of specific caspase substrates and cell death [7,10]. Because caspase activities can be detected in all cells undergoing apoptosis, regardless of their origin or death stimuli, and inhibitors of caspases prevent the hallmarks of apoptosis, these proteases are considered the main executioners of apoptosis [13,14].

Due to the relevance of the potential anti MDR-activity of PA and considering that characterization of their mechanisms of action is a preliminary and fundamental step to future clinical application/use of new drugs, the aim of this paper is to clarify the mechanism of action of this triterpene. Investigation of PA-induced death pathways demonstrated that induction of apoptosis by this triterpene involves activation of caspases-9 and -3 in a process mediated through loss of $\Delta\psi_m$, an unusual mechanism for drug-induced apoptosis in HL-60 cells. Indeed, treatment of cells with CSA an inhibitor of mitochondria PTP, completely abrogates apoptosis suggesting that mitochondria is the main target for PA. Since it has been proposed that drugs that act on mitochondria may bypass MDR resistance mechanisms, our data reinforce the potential of pomolic acid as a new anti-MDR drug.

2. Materials and methods

2.1. Chemicals and cell culture

Pomolic acid isolated from *Chrysobalanus icaco*L. as described previously [1] was dissolved in dimethyl sulfoxide (DMSO, SIGMA, St Louis) and diluted in RPMI 1640 for use. $DiOC_6(3)$ was from Molecular Probes, Inc. (Eugene, OR), cyclosporin A was from Calbiochem (San Diego, CA, USA), caspase inhibitor zVAD-fmk was from Enzyme Systems (Livermore, USA), mAbs for caspases-3 and -9 were from Pharmingen (San Diego, CA, USA), anti-actin mAb was from ICN (Costa Mesa, USA) and anti-mouse IgG-HRP from Amersham Biosciences (Arlington, IL). The human leukemia cell line HL-60 was cultured at 37 °C and 5% CO_2 in RPMI 1640 (Life Technologies, Inc., USA) supplemented with heat inactivated 10% fetal calf serum (FCS; Life Technologies, Inc., USA), 2 mM L-glutamine, 100 U/ml of penicillin and 100 μg/ml of streptomycin (Life Technologies, Inc., USA).

2.2. Cell viability assay

Cell viability was assessed by MTT [15]. Cells (10^4/well) were plated in 96 well tissue culture plates for 24 h and then treated with different concentrations of PA (1, 10, 25, 100 μg/ml) or DMSO (in the same concentrations carried by the drug). After another 48 h, 20 μl (5 mg/ml) MTT was added to each well and the plate was incubated at 37 °C in the dark for at least 4 h. The formazan crystals were solubilized in DMSO (200 μl/well) and the reduction of MTT was quantified by absorbance (*A*570 nm, with 630 nm-reference filter). Effects of the drug on cell viability were calculated using cells treated with DMSO as control.

2.3. Detection of apoptosis

Apoptosis was evaluated by cell morphology and cell cycle analysis [16]. After 24 h resting, plated cells