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Combination of ABT-737 and resveratrol enhances DNA damage and apoptosis in human T-cell acute lymphoblastic leukemia MOLT-4 cells

Małgorzata Opydo-Chanek[a,*], Agnieszka Rak[b], Agnieszka Cierniak[c], Lidia Mazur[a]

[a] Department of Experimental Hematology, Institute of Zoology, Jagiellonian University, Gronostajowa 9, 30-387 Kraków, Poland
[b] Department of Physiology and Toxicology of Reproduction, Institute of Zoology, Jagiellonian University, Gronostajowa 9, 30-387 Kraków, Poland
[c] Department of General Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland

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ABSTRACT

ABT-737 belongs to a new class of anticancer agents named BH3 mimetics. ABT-737 competitively binds to surface hydrophobic grooves of anti-apoptotic proteins of Bcl-2 family, counteracting their protective effect. Resveratrol is a natural polyphenol that has been shown to inhibit the proliferation and/or induce apoptosis in a number of different types of cancer cells. The present study was designed to analyze the combined effects of ABT-737 and resveratrol on human acute lymphoblastic leukemia cells. The in vitro cytotoxic activity of these agents against MOLT-4 leukemia cells was determined using the Coulter electrical impedance method, comet assay, and flow cytometry, light microscopy and western blot techniques. The results are the first data showing that ABT-737 combined with resveratrol markedly decreased the cell viability, increased DNA damage, caused the cell cycle perturbation, and synergistically enhanced apoptosis in MOLT-4 cells, when compared to the data obtained after application of the single agent. Moreover, the simultaneous treatment of leukemia cells with ABT-737 and resveratrol resulted in a reduction in mitochondrial membrane potential, an increase of p53 protein level and up-regulation of the Bax/Bcl-2 ratio. The obtained data indicate that the combination of ABT-737 and resveratrol is a promising approach for acute lymphoblastic leukemia treatment that should be further explored.

1. Introduction

T-cell acute lymphoblastic leukemia (T-ALL) represents a malignant disorder arising from T-cell progenitors. T-ALL accounts for 10–15% of pediatric and 25% of adult newly diagnosed ALL cases. Although the prognosis of T-ALL has gradually improved with the introduction of intensified chemotherapy, the outcome of T-ALL patients with primary resistant or relapsed leukemia remains poor (Pui et al., 2008). Therefore, there is an urgent need to develop more effective and less toxic treatments for T-ALL patients.

T-ALL cells are characterized by unlimited self-renewal, uncontrolled cell cycle progression, impaired differentiation and loss of sensitivity to apoptosis (Pui et al., 2008). Cell proliferation and apoptosis are believed to be closely linked and regulated, and a failure of these mechanisms determines profound dysregulation of cellular homeostasis (King and Cidlowski, 1995). The evasion of apoptosis through aberrant expression of Bcl-2 family proteins is a common feature of T-ALL. Elevated levels of the anti-apoptotic members of Bcl-2 family has been reported in acute lymphoblastic leukemia cell lines and primary samples (Campana et al., 1993; Coustan-Smith et al., 1996; Del Gaizo Moore et al., 2008). Over-expression of these proteins influences the survival ability of leukemic lymphoblasts and can contribute to leukemogenesis and chemoresistance (Tzifi et al., 2012). Thus, the anti-apoptotic proteins of Bcl-2 family represent an attractive therapeutic target for T-ALL treatment. ABT-737 is a small-molecule Bcl-2 inhibitor that belongs to a new class of anticancer agents known as BH3 mimetics. ABT-737 has been rationally designed based on structural information gathered on protein–protein interactions within the Bcl-2 family. This agent mimics the pro-apoptotic BH3-only protein Bad by binding with high affinity to Bcl-2, Bcl-xL, and Bcl-w and is referred to as Bad-like BH3 mimetic (Konopleva et al., 2006; Oltersdorf et al., 2005; van Delft et al., 2006). Previous studies have shown that ABT-737 is a highly promising anti-cancer agent (Del Gaizo Moore et al., 2008; High et al., 2010). Moreover, this BH3 mimetic significantly potentiates

Abbreviations: T-ALL, T-cell acute lymphoblastic leukemia; RES, resveratrol; Bcl-2, B-cell lymphoma 2; Bcl-xL, B-cell lymphoma extra-large; Bcl-w, BCL-2-like protein 2; Bax, Bcl-2-associated X protein; Bak, Bcl-2-associated killer; Bad, Bcl-2-associated death promoter; Bim, Bcl-2-like protein 11; BH, Bcl-2 homology domain; DMSO, dimethyl sulfoxide; HBSS, Hank's balanced salt solution; TMRE, tetramethylrhodamine ethyl ester perchlorate; FDA, fluorescein diacetate; PI, propidium iodide; annexin V-FITC, fluoresceinated annexin V; MMP, mitochondrial membrane potential; γ-H2AX, gamma-H2A histone family member X; ATM, ataxia telangiectasia mutated

* Corresponding author.
E-mail address: malgorzata.opydo-chanek@uj.edu.pl (M. Opydo-Chanek).

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the efficacy of established and novel anticancer drugs (High et al., 2010; Kuroda et al., 2008; Reynoso et al., 2011). The orally bioavailable analog of ABT-737, ABT-263, is currently in phase I/II clinical trials as a single agent and in combination therapies for lymphoid malignancies, chronic myelogenous leukemia, and solid tumours (Gandhi et al., 2011; Kipps et al., 2015; Roberts et al., 2012).

Resveratrol (3,5,4′-trihydroxystilbene, RES) is a natural polyphenol belonging to the class of stilbenes. RES is a nontoxic phytoalexin synthesized de novo by several plants in response to stress, injury, UV irradiation and pathogen infections (Singh et al., 2015). Recently, the high efficacy of RES against various cancers has been shown in both in vitro and in vivo studies (Joe et al., 2002; Li et al., 2010; Zunino and Storms, 2006), and clinical trials have been initiated using resveratrol as an anticancer oral compound (Bishayee, 2009; Singh et al., 2015). Considerable efforts have been done to explore anticancer potential of resveratrol via combining this polyphenol with other anticancer agents, in order to increase the overall therapeutic efficacy (Singh et al., 2013).

Combined application of Bcl-2 inhibitors and natural origin compounds may represent a promising therapeutic approach toward improvement of the effectiveness of cancer therapy. Previous studies indicated that both ABT-737 and resveratrol, applied as a single agent, are potent apoptosis inducer in cancer cells. Inhibition of anti-apoptotic proteins of Bcl-2 family and consequently activation of mitochondrial apoptotic pathway are well-known phenomena of ABT-737 anticancer action (Konopleva et al., 2006; van Delft et al., 2006). Among various mechanisms proposed to explain the anticancer activity of resveratrol, induction of DNA damage that leads to the activation of the p53 pathway and apoptosis has been well established (Ferraz da Costa et al. 2012; Podhorecka et al. 2011). Moreover, it was shown that resveratrol altered the expression of both pro- and anti-apoptotic members of Bcl-2 family (Podhorecka et al. 2011). Thus, ABT-737 and resveratrol activate the mitochondrial pathway of apoptosis by distinct but complementary mechanisms. Based on these observations, we hypothesized that combination of ABT-737 and resveratrol may potentiate cytotoxicity by enhancing apoptosis in cancer cells. Therefore, the present study was designed to investigate the cytotoxic effects of the combination of ABT-737 and RES on T-cell acute lymphoblastic leukemia MOLT-4 cells. It is known that DNA damage, alterations in the level of p53 and Bcl-2 family proteins as well as changes in mitochondria can trigger apoptotic cell death and/or cause the cell cycle perturbation. Thus, to obtain insights into mechanisms of the combined action of ABT-737 and RES, their influence on the leukemia cell viability, DNA damage, cell cycle distribution, apoptosis induction, mitochondrial membrane potential, and on the level of p53, Bcl-2 and Bax proteins was examined.

2. Material and methods

2.1. Reagents

ABT-737 was purchased from Selleck Chemicals (Munich, Germany), dissolved in DMSO and stored as 5 mM stock solutions at − 20 °C. Resveratrol was purchased from Sigma Aldrich (St. Louis, MO, USA). A RES stock solution was prepared at a concentration of 30 mM in DMSO directly before treatment of cells. The final concentration of RES was obtained by diluting this stock solution in culture medium. RPMI 1640 medium, fetal calf serum and Hank's balanced salt solution (HBSS) were from GIBCO BRL Life Technologies (Gaithersburg, MD, USA). L-glutamine, antibiotic antimycotic solution (AAS), dimethyl sulfoxide (DMSO), fluorescein diacetate, tetramethylrhodamine ethyl ester perchlorate (TMRE), anti-β-actin antibody (cat. #A5316) were purchased from Sigma Aldrich. FITC Annexin V Apoptosis Detection Kit I and APO-BrdU™ kit were obtained from BD Pharmingen (BD Biosciences, San Diego, CA). CellEvent™ Caspase 3/7 Green Flow Cytometry Assay kit was purchased from Molecular Probes (Eugene, OR, USA). The Hemacolor® Rapid Staining kit was obtained from Merck Millipore (Darmstadt, Germany). Antibodies against Bax (cat. #2772), Bcl-2 (cat. #2876), p53 (cat. #9282) and horseradish peroxidase-conjugated secondary antibody (cat. #7074) were purchased from Cell Signalling Technology (Danvers, MA, USA).

2.2. Cell line, culture and treatment

Human acute lymphoblastic leukemia MOLT-4 cells were obtained from European Collection of Cell Cultures (Salisbury, UK). MOLT-4 cells were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM L-glutamine and AAS containing 20 units of penicillin, 20 mg streptomycin and 0.05 mg amphotericin B. Cells were passaged every third day. The cells grew exponentially at 37 °C in an atmosphere of 5% CO_2 in air (HERAcell incubator). Leukemia cells were seeded in 12- or 24-well plates, at a density of 15×10^4 cells/mL, prior to performing experiments. MOLT-4 cells were treated with ABT-737, at two concentrations, 0.25 μM and 0.5 μM, and/or RES, at a concentration of 30 μM. The concentrations of the tested agents were chosen based on preliminary in vitro studies. These concentrations are physiologically relevant and may be achieved in vivo. The controls consisted of untreated and DMSO-treated leukemia cells. The final concentration of DMSO did not influence the analyzed parameters, and no significant differences in the cell response to this diluent were observed. Thus, the data obtained for DMSO-treated cells are considered the control. The influence of ABT-737 and RES, given alone and in combination, on leukemia cells were determined 24 h and/or 48 h after the tested agent application.

2.3. Cell viability assay

The influence of ABT-737 and RES on the leukemia cell viability was analyzed using the flow cytometry fluorescein diacetate (FDA)/propidium iodide (PI) assay as previously described in detail (Opydo-Chanek et al., 2014). FDA and PI fluorescence was measured using FACS Calibur flow cytometer (Becton Dickinson). The frequency of live cells (FDA + /PI-) were analyzed using WinMDI 2.8 software. The cell viability was expressed as percentage of the control value.

2.4. Coulter counter measurements

The MOLT-4 leukemia cell volume and count were analyzed using a Z2 Coulter counter (Beckman Coulter, USA) as previously described in detail (Mazur et al. 2013). The mean cell volume and also the cell count were determined at a range 481.1–3622 fl, using Z2 AccuComp software (Beckman Coulter, USA).

2.5. Comet assay

Cell suspension in low melting point agarose was placed on a microscope slides covered previously with normal melting point agarose. Then, the slides were covered with cover glass and put on ice for 5 min. Afterward, the cover glass was removed. Cells were lysed in a solution of 2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100 and 10% DMSO, pH 10. Lysis was performed in the dark at 4 °C for 1 h. Then, slides were rinsed twice with 0.4 M Tris, pH 7.5, and placed on the horizontal gel box filled with buffer containing 1 mM EDTA and 300 mM NaOH, pH > 13. Slides were left in the dark at 4 °C for 40 min. After this time, the slides were subjected to electrophoresis at 0.74 V/cm, 300 mA, for 30 min. After electrophoresis, the slides were rinsed three times with 0.4 M Tris, pH 7.5, and exposed to cold 100% methanol for 5 min. Directly before analysis, the slides were immersed in redistilled water for 5 min, and stained with propidium iodide, at the concentration of 2.5 μg/ml. To visualize DNA damage, epifluorescence microscope (Olympus IX-50) with CCD camera was used. The analysis of DNA damage was conducted using COMET ASSAY 2.9 software (Comet Plus, Theta System Gmbh, Germany). For each sample, 100