RESEARCH ARTICLE

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Dihydroartemisinin Enhances the Therapeutic Efficacy of BH3 Mimetic Inhibitor in Acute Lymphoblastic Leukemia Cells via Inhibition of Mcl-1

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Abstract

Introduction: Up-regulation of the anti-apoptotic proteins such as Mcl-1 is associated with the primary and secondary resistance of tumor cells to ABT-737 Bcl-2 inhibitor. The combined treatment of Bcl-2 inhibitors with Mcl-1 inhibitors has been proposed as an attractive therapeutic strategy to overcome this drug resistance. Here, we investigated the effect of dihydroartemisinin on Mcl-1 expression and sensitization of T-ALL cells to ABT-737. **Methods:** The cell growth and survival were tested by the cell proliferation and MTT assays, respectively. The mRNA levels of Bcl-2, Mcl-1, Bax and P21 were examined by qRT-PCR. Apoptosis were detected by Hoechst 33342 staining and caspase-3 activity assay. **Results:** Our data showed that combination treatment with dihydroartemisinin and ABT-737 caused a significant decrease in the IC_{50} value and synergistically reduced the cell survival compared with dihydroartemisinin or ABT-737 alone. ABT-737 enhanced the Mcl-1 mRNA expression. Dihydroartemisinin also down-regulated the expression of Bcl-2 and Mcl-1 and enhanced the P21 and Bax expression. Moreover, dihydroartemisinin enhanced the apoptosis induced by ABT-737 in MOLT-4 and MOLT-17 cell lines. **Conclusion:** In conclusion, dihydroartemisinin demonstrates anti-tumor activities in human ALL cells via inhibition of cell survival and growth. Dihydroartemisinin augments the apoptotic effect of ABT-737 by inhibiting the expression of Mcl-1.

Keywords: ABT-737, ALL, Apoptosis, Dihydroartemisinin, Mcl-1

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Introduction

Acute lymphoblastic leukemia (ALL) represents a heterogeneous malignant disorder arising from hematopoietic blasts [1]. Although the survival rate of ALL patients has improved with the introduction of high-dose chemotherapy, almost half of children and adult patients invariably relapse. Therefore, there is an urgent need to understand the underlying molecular-resistance mechanisms and more effective treatment strategies for ALL patients [1-3].

Cell apoptosis is regulated by two groups of pro- and anti-apoptotic proteins. The interaction of these two groups determines the cell survival or cell apoptosis [4]. Overexpression of Bcl-2 family anti-apoptotic proteins has been observed in ALL cell lines and primary samples, which is associated with increased in cell growth and cell cycle, reduced apoptosis as well as resistance to chemical drugs. Therefore, targeting these proteins has been proposed as an attractive strategy for the treatment of T-ALL [5-8].

ABT-737 is a BH3 mimic drug designed and developed based on bioinformatics data of protein-protein interaction. This drug binds to Bcl-2, Bcl-xL and Bcl-w anti-apoptotic proteins with high affinity and to Mcl-1 protein weakly [5, 9, 4]. ABT-737 not only shows high single anticancer agent activity, but also enhances the efficiency of other anticancer drugs [9]. Various studies show that up-regulation of the anti-apoptotic protein Mcl-1 increases the primary and secondary resistance of various tumor cells such as ALL to ABT-737. Therefore, combined treatments including Bcl-2 inhibitors with other Mcl-1 inhibiting compounds have been proposed as an attractive therapeutic strategy [10-13].

Artemisinin is an active phytochemical from the Artemisia annua plant that is used worldwide as an

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effective antimalarial drug. In addition, artemisinin and its derivatives have antitumor properties [14]. Dihydroartemisinin is one of the derivatives of artemisinin that has shown the strongest anticancer effects compared to other derivatives of this group. This compound changes the oxidative stress response, DNA damage and repair system, cell cycle mechanism, different states of cell death and angiogenesis via different cell signaling pathways such as MAPK, B-catenin, NF-kB and mTOR [14]. In addition, the results of various studies show that dihydroartemisinin and its derivatives cause cell cycle arrest and induce apoptosis through the induction of oxidative stress [14-16]. These effects of dihydroartemisinin are carried out by changing the expression of genes effective in cell cycle and apoptosis, such as cyclins, P21, Bcl-2, Bax and Mcl-1 [14, 16]. However, the effect of dihydroartemisinin on sensitivity to ABT-737 is still unclear. In this study, the effect of dihydroartemisinin on Mcl-1 expression and sensitization of T-ALL cells to ABT-737 was investigated.

Materials and Methods

Cell culture

The human T-ALL cells lines MOLT-4 and MOLT-17 were purchased from the Pasteur Institute of Iran (Tehran, Iran). MOLT-4 and MOLT-17 cells were grown in RPMI-1640 medium (Sigma-Aldrich, St. Louis, MO, USA) containing 20% heat-inactivated fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA, USA). All cell cultures were maintained at 37°C in a humidified atmosphere with 5% CO_2.

MTT cell survival assay

The MTT assay was performed to measure the effects of dihydroartemisinin and ABT-737, alone and in combination, on survival of the leukemic cells. The assay was divided into 5 groups: blank control, solvent control, dihydroartemisinin, ABT-737 and dihydroartemisinin+ABT-737. Briefly, the cells were seeded in 96-well culture plates an initial density of 2×10^4 cells per well. Then, the cells were treated with different concentrations of dihydroartemisinin and ABT-737 and were allowed to incubate for 24 h. Next, 10 µl of MTT solution (Sigma-Aldrich) (5 mg/ml) was added to each well. After 4 h of incubation at 37 oC, the mediums were removed, and 150 µl of DMSO was added to wells. Absorbance (A) at 490 nm was quantified with an ELISA reader (Awareness Technology, Palm City, FL, USA). The survival rate (SR) was determined from the following formula: SR (%) = $(A_{Test}/A_{Control}) \times 100\%$. IC_{50} (the concentration that reduced 50% of cell survival) was calculated by GraphPad software (GraphPad Software Inc., San Diego, CA, USA). In the next experiments the IC_{50} doses of drugs were used.

Analysis of combined effect

To further study the effect of combination treatment therapy; the combination index (CI) analysis based on the principles established by Chou and Talalay method [17, 18] was performed. The cell survival values of MTT test were converted to Fraction affected (Fa; where Fa = 0 is 100% cell survival and Fa = 1 is 0% cell survival) and analyzed by CompuSyn software (ComboSyn Inc., Paramus, NJ, USA). CI values reflect the ways of interaction between ABT-737 and dihydroartemisinin. CI=1 indicates an additive effect, CI<1 indicates synergism effect, and CI>1 indicates antagonism effect.

Cell growth assay

The effect of dihydroartemisinin and ABT-737 on cell proliferation was assessed by the trypan blue exclusion assay. Briefly, 5×103 leukemic cells were treated with ABT-737 and dihydroartemisinin, alone and in combination, in 6-well plates for five days. At different times, total cells were harvested and cell suspensions stained with 0.4% trypan blue (Merck KGaA, Darmstadt, Germany) for 2 min. Subsequently, the number of viable cells (unstained cells) was counted using a hematocytometer and an inverted microscope (Nikon Instrument Inc., Melville, NY, USA). The percentage of unstained cells was determined by the equation as follows: Cell viability (%) = $(N_{Test}/N_{Control}) \times 100$. The viability of blank control cells at indicated time was considered as 100%.

qRT-PCR

At various time points after treatment, total RNA was isolated by RNA extraction reagent (Parstous, Tehran, Iran) following the manufacturer's protocol. Complementary DNA (cDNA) was synthesized from 1 µg of total cellular RNA using MMLV reverse transcriptase (Parstous) and oligo-dT primer according to the manufacturer's instructions. Reverse transcription polymerase chain reaction (RT-PCR) was quantified by the LightCycler 96 system (Roche Diagnostic GmbH, Mannhein, Germany) by SYBR Premix (Parstous). The reaction system of real-time PCR was: 0.2 µM of each primer, 12 µl of STBY green PCR Master Mix, 1 µl of cDNA template, and 6 µl of nuclease-free distilled water. The specific primers used for PCR analysis are listed in Table 1. The initial denaturation qRT-PCR step at 95˚C for 10 min was followed by 35 cycles at 95˚C for 20 sec and 60˚C for 1 min. The relative expression level of mRNA was analyzed with the $2^{-(\Delta\Delta Ct)}$ method [19, 20], by using β-actin as an endogenous control gene.

Hoechst staining

The human T-ALL cell lines were exposed to IC_{50} concentration of dihydroartemisinin and ABT-737, alone and in combination, for 24 h. Next, the T-ALL cells were washed with PBS and fixed in 4% formaldehyde for 30 min at room temperature. Subsequently, 5 µg⁄mL of Hoechst 33342 (Sigma-Aldrich) was used to stain the cells for 30 min. The nuclear morphological changes were observed under a fluorescence microscope (Olympus, Tokyo, Japan).

Caspase-3 activity assay

The in vitro caspase-3 activity was measured using a caspase activity assay Kit (Abnova Corporation, Taipei, Taiwan). The treated cells were lysed in lysis buffer and the cell suspensions were centrifuged in 12,000 g for 3 min. Then, 50 µg of supernatant and 50 µl of 3 reaction

buffer was transferred to a new tube. Then, the caspase-3 colorimetric substrate with a final concentration of 200 μM was added into each well and incubated for 2 h at 37°C. The absorbance was determined at 405 nm.

Statistical analysis

All data in this study were presented as mean ± standard deviation (SD). Statistical significance of differences between groups was assessed by using analysis of variance (ANOVA) and t-test using GraphPad Prism software. P-value less than 0.05 were considered significant.

Results

Dihydroartemisinin increased the sensitivity of ALL cells to ABT-737

The effects of dihydroartemisinin and ABT-737, alone and in combination, on ALL cell survival were investigated using MTT assay. The results showed that mono-therapy with each of these agents significantly decreased the cell survival rate in a dose-dependent way (relative to the blank control) (Figure 1). The IC_{50} values of dihydroartemisinin and ABT-737 for 24 h treatment were 62.16, 1.49 μM in MOLT-4 cells, and 45.37 and 1.21 μM in MOLT-17 cells, respectively (Table 2). Moreover, the dihydroartemisinin in combination with ABT-737 further lowered the cell survival rate and reduced the IC_{50} value, relative to the monotreatment ($p<0.05$). These results suggest that dihydroartemisinin can inhibit the cell survival rate and also enhance the sensitivity of the T-ALL cells to the ABT-737.

The combination effect of dihydroartemisinin and ABT-737 on leukemic cells was synergistic

To investigate whether the effect of dihydroartemisinin with ABT-737 on survival of the T-ALL cells is responsible for their synergistic interaction, we performed the CI

Table 1. Sequence of Primers Used in Real-Time PCR

Genes	Sequence (5'→3')
β-actin FW	GACATCCGCAAAGACCTGTA
β-actin RV	GGAGCAATGATCTTGATCTTCA
Bcl-2 FW	GGATGCCTTTGTGGAACTG
Bcl-2 RV	CAGCCAGGAGAAATCAAACAG
Bax FW	GCTTCAGGGTTTCATCCAG
Bax RV	TTACTGTCCAGTTCGTCCC
P21 FW	TGGAGACTCTCAGGGTCGAAA
P21 RV	CGGCGTTTGGAGTGGTAGAA
Mcl-1 FW	TAGTTAAACAAAGAGGCTGGGA
Mcl-1 RV	CCTTCTAGGTCCTCTACATGG

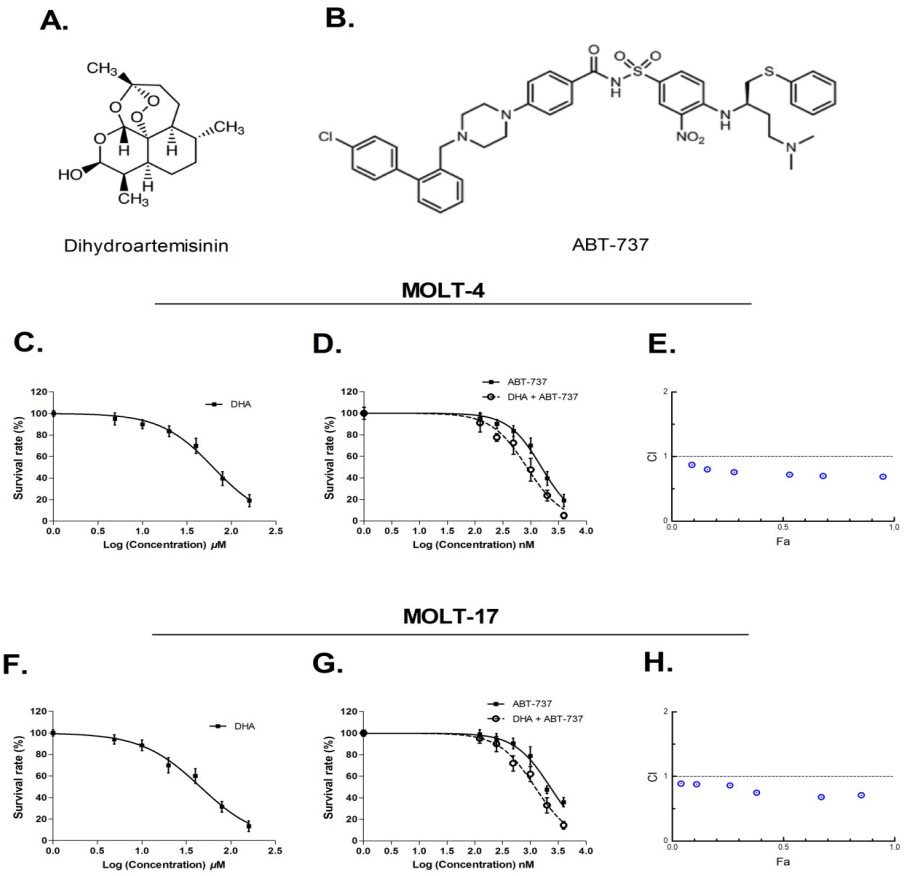

Figure 1. Effect of Dihydroartemisinin and ABT-737 on Cell Survival. The MOLT-4 and MOLT-17 were treated with dihydroartemisinin (DHA) (A) and ABT-737 (B) at indicated concentrations. After 24 h, the cell survival rate was determined using MTT assay. The cell survival curves were plotted using GraphPad 6.1 software (C, D, F and G). Data are expressed as mean±SD of three experiments. The combination index (CI) values were determined using the fractional affected (Fa) values of MTT assay and CalcuSyn software (E and H).

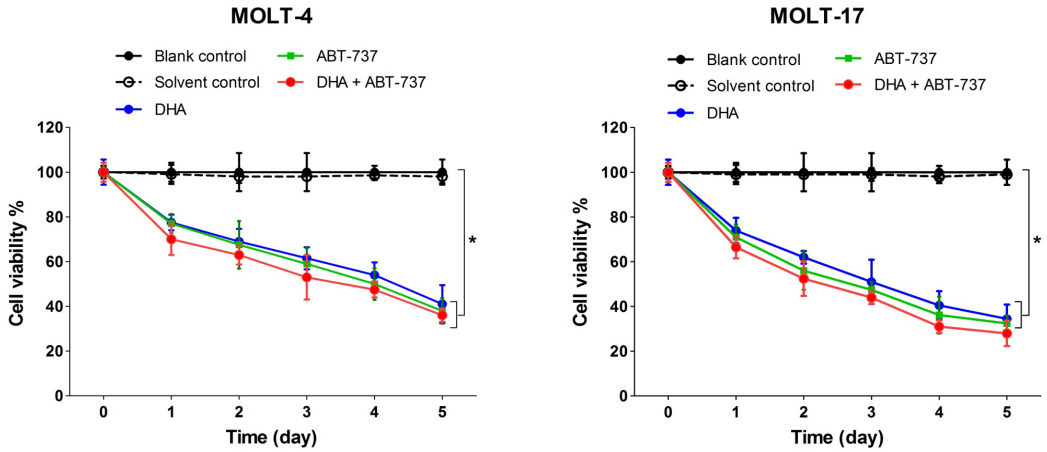

Figure 2. Growth Inhibition of ALL Cells. The MOLT-4 and MOLT-17 cells were treated with ABT-737 and dihydroartemisinin (DHA) for 1-5 day, and the cell viability was determined using trypan blue exclusion assay at the end of each day. The data are expressed as mean±SD of three independent experiments. *p<0.05 versus blank control or solvent control.

Table 2. IC_{50} Values of the ABT-737 alone and in Combination with Dihydroartemisinin in MOLT-4 and MOLT-17 ALL Cells.

	IC50 (24 h)	
	MOLT-4	MOLT-17
ABT-737	1.49	2.27
Dihydroartemisinin	62.16	45.37
Combination	0.85*	1.21*

IC_{50} was calculated using sigmoidal dose-response model and Prism software. Data are expressed as the mean±SD (n=3). *p<0.05, versus single treatment.

analysis using CompuSyn software. The CI–Fa plots showed that the combination effect of dihydroartemisinin (0–4 μM) and ABT-737 (0–4 μM) was synergistic (CI<1) in all of concentrations. The result of CI analysis is shown in full in Figure 1.

Dihydroartemisinin enhanced the growth inhibitory effect of ABT-737

We then investigated whether dihydroartemisinin and ABT-737 could inhibit the growth of T-ALL cells. The cells were treated with dihydroartemisinin and ABT-

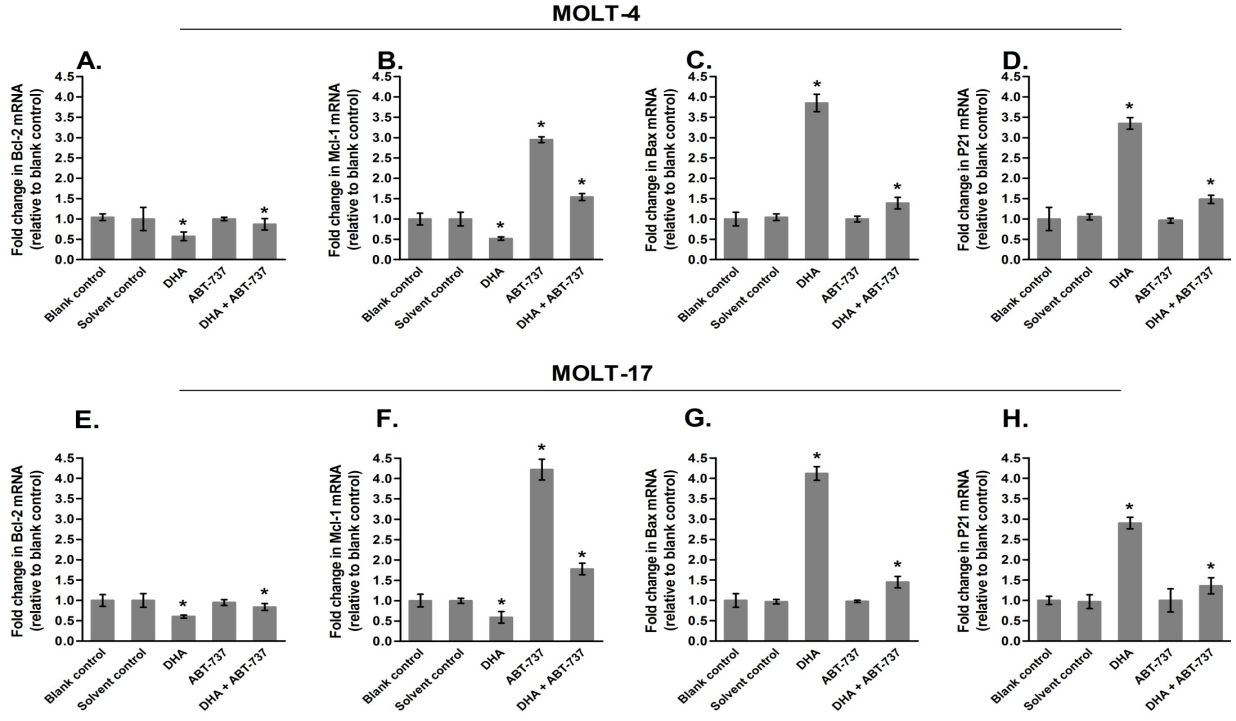

Figure 3. RT-qPCR Analysis of ALL Cells. The MOLT-4 and MOLT-17 cells were treated with ABT-737 and dihydroartemisinin (DHA) (IC50 doses). After 24 h, relative mRNA expression levels of Bcl-2 (A and E), Mcl-1 (B and F), Bax (C and G) and P21 (D and H) were measured by RT-qPCR using 2-(ΔΔCt) method. Data are presented as mean±SD (n=3). *p<0.05, relative to blank control.

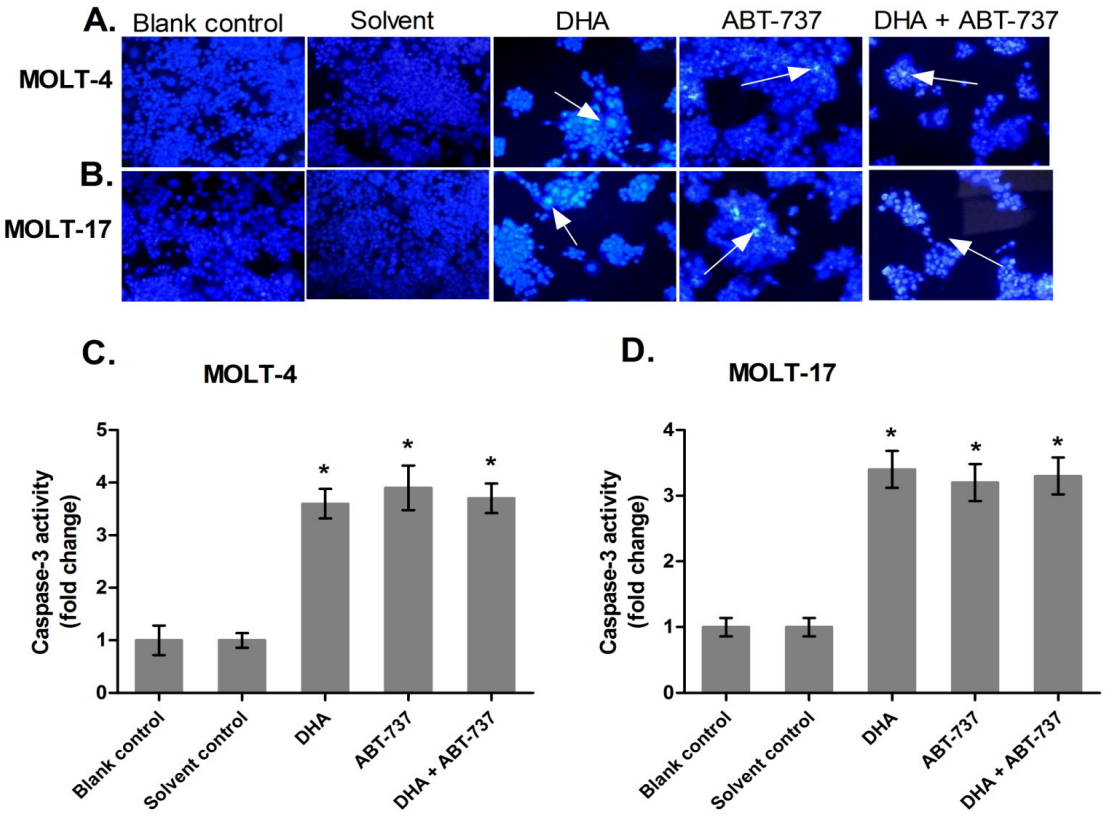

Figure 4. Combination Effects of ABT-737 and Dihydroartemisinin on Apoptosis of ALL Cells. MOLT-4 and MOLT-17 cells were treated with the IC50 doses ABT-737 and dihydroartemisinin (DHA) for 24 h. Next, the apoptosis was measured using Hoechst 33342 staining (A and B) and caspase-3 activity assay (C and D). The results are expressed as mean±SD (n=3) of three experiments. *p<0.05, compared with blank control. Arrows show the apoptotic cells.

737, alone and in combination and the cell viability was measured by trypan blue dye exclusion assay during a period of 120 h. The results were stated as the percentage of viable cells in relation to the total number of cells. The cell growth curve showed that compared with the blank control and solvent control groups, the percent of viable cells in dihydroartemisinin, ABT-737 and combinatorial group markedly decreased time-dependently. In MOLT-4 cells, the cell growth in dihydroartemisinin, ABT-737 and combinatorial group dropped to 78 %, 77 % and 69 % respectively, after 24 h of treatment, and then to a further 41 %, 38 % and 36 %, respectively, at the end of the fifth day (p<0.05;Figure 2). Similar results were observed in the MOLT-17 cell line.

Formononetin changed the expression of apoptotic proteins

We used the qRT-PCR method to explore the effect of dihydroartemisinin and ABT-737 on mRNA levels. The results showed that after 24 h of treatment of ALL cells with dihydroartemisinin, the expression levels of Mcl-1 and Bcl-2 decreased significantly compared to the blank control group, while the expression levels of P21 and Bax increased. Treatment with ABT-737 did not cause a significant change in the mRNA levels of P21, Bax and Bcl-2, while the expression of Mcl-1 mRNA increased (compared to the blank control group and Figure 3). In the combination group, a marked decrease in the expression of Bcl-2 and increase in the expression of P21 and Bax were observed compared to the ABT-737 and blank control groups (Figure 3, p<5%). These changes were significantly less than dihydroartemisinin group. In addition, there was a significant difference in Mcl-1 mRNA expression levels between the combination group with the single treatment and blank control groups (Figure 3, p<5%). In the solvent control group, there was no clear difference in gene expression compared to the blank control group (p>5%). The results obtained were consistent across both MOLT-4 and MOLT-17 cell lines.

Formononetin enhanced the apoptotic effect of ABT-737 in leukemic cells

To explore whether the cytotoxicity of treatments was related to apoptosis, the MOLT-4 and MOLT-17 cells were exposed to the IC_{50} doses of dihydroartemisinin, ABT-737 and their combination for 24 h as described before. Next, we performed Hoechst 33342 staining and caspase-3 activity assay. Nuclear morphological change is one of the most common characteristics of cell death. So, Hoechst 33342 staining was used to show apoptotic morphologic changes in ALL cells. As shown in Figure 4, apoptotic cells containing nuclear fragments were observed in dihydroartemisinin and ABT-737 treated cells but not in the control cells. Furthermore, the number of apoptotic cells in the combination treatment group did not significantly differ from the mono-treated cells.

To understand the molecular mechanism of apoptosis triggered by dihydroartemisinin and ABT-737, caspase-3 activity assay was performed. The results showed that there was a significant increase in caspase-3 activity in the cells treated with dihydroartemisinin and ABT-737. The percentage of caspase-3 activity in the ALL cells treated with the combination of dihydroartemisinin and ABT-737 was not different relative to caspase-3 activity in the cells treated with either dihydroartemisinin or ABT-737 alone ($p>0.05$, Figure 4).

Because the IC_{50} dose of the combination group is lower than the IC_{50} dose of either compound alone, thus, our data demonstrate that the combination of the two agents has a greater effect on triggering apoptosis compared to treatment with each single agent. The enhanced apoptotic effect with the combination is consistent with the results from the MTT assay.

Discussion

The survival rate of ALL patients has improved with the introduction of high-dose chemotherapy. However, almost half of children and adult patients still experience relapse. This highlights the need for a better understanding of the underlying molecular-resistance mechanisms and the development of more effective treatment strategies for ALL patients [1-3]. Overexpression of Bcl-2 family anti-apoptotic proteins is associated with increased cell growth, cell cycle, reduced apoptosis, and resistance to chemical drugs in ALL cell lines and primary samples [5, 6, 21, 8]. Mcl-1 is an anti-apoptotic protein that increases primary and secondary resistance of various tumor cells, including ALL, to ABT-737. Therefore, combined treatments including Bcl-2 inhibitors with other Mcl-1 inhibiting compounds have been proposed as an attractive therapeutic strategy [22, 11-13]. In this study, the effect of dihydroartemisinin on Mcl-1 expression and sensitization of T-ALL cells to ABT-737 was investigated.

The results of our study revealed that treatment with either ABT-737 or dihydroartemisinin alone significantly decreases the cell proliferation and survival and induces cell death. Combination treatment with dihydroartemisinin and ABT-737 caused a significant decrease in the IC_{50} value and synergistically reduced the cell survival compared with dihydroartemisinin or ABT-737 alone. The IC_{50} dose of the combination was lower than the IC_{50} dose of either agent alone; therefore our results suggest that the combination therapy has a greater effect on cell proliferation and apoptosis compared to treatment with monotherapy. So far, several studies have shown the relationship between the expression level of Mcl-1 and resistance to ABT-737 in tumor cells. Tahir et al. [11] investigated the contribution of Bcl-2 family proteins to the cellular response of several lung cancer cell lines to ABT-737. They found that higher expression of Bcl-2, Bcl-xL, Bim, and Noxa, and lower expression of Mcl-1 are characterized lung cancer cell lines that are sensitive to ABT-737. In a study conducted by Wangand Hao [23] they showed that A-1210477, a specific Mcl-1 inhibitor, could overcome resistance to ABT-737 in AML cells that overexpressed Mcl-1. The combination of A- ABT-737 and 1210477 enhanced apoptosis in AML cells. In another study, Konopleva et al. [4] explored the underlying mechanisms of resistance and sensitivity of AML cells to the ABT-737. They found that AML cells with high levels of Bcl-xL, Mcl-1 or Bcl-2 were more resistant to ABT-737. Moreover, activation of PI3K/Akt pathway conferred resistance to ABT-737. Yecies et al. [22] showed that lymphoma cells with resistance to ABT-737 overexpressed the Mcl-1 and, combination of Mcl-1 inhibitors with ABT-737 could overcome this resistance. Here, we showed that dihydroartemisinin lowers the expression level of Mcl-1 mRNA and enhances the apoptosis induced by ABT-737 in MOLT-4 and MOLT-17 cell lines. Our data are in agreement with the above reports and indicate that dihydroartemisinin can increase the sensitivity of the ALL cells to ABT-737 by inhibiting the expression of Mcl-1.

We also explored the effect of dihydroartemisinin and ABT-737 on mRNA expression. The qPCR assay data revealed that ABT-737 enhances the Mcl-1 mRNA expression without affecting the of P21, Bcl-2 and Bax expression. Dihydroartemisinin also down-regulated the expression of Bcl-2 and Mcl-1 and enhanced the P21 and Bax expression. In combination treatment, dihydroartemisinin lowered the Mcl-1 expression induced by ABT-737, which was related with increased sensitivity to ABT-737. In accordance with our study, several researches have been conducted regarding the effect of dihydroartemisinin on gene expression and chemoresistance. Hou et al. [24] in a study showed that dihydroartemisinin exerts great cytotoxicity in hematoma cells but significantly lowers cytotoxicity in normal liver cells. This compound inhibited cell proliferation, induced cell cycle arrest, decreased the levels of cyclin D1, cyclin E, and increased the levels of Cip1/p21 and Kip1/p27. Dihydroartemisinin also increased the Bax/Bcl-2 ratio and induced apoptosis. Moreover, it enhanced the efficacy of the chemotherapeutic agent gemcitabine. Results of another study revealed that treatment of Jurkat T-lymphoma cells with dihydroartemisinin lead to release of cytochrome c, activation of caspases, DNA fragmentation and induction of apoptosis. Furthermore, dihydroartemisinin treatment induced the expression of Noxa and activated the Bak protein. Dihydroartemisinin also increased the cytotoxic effect of ionizing radiation in Jurkat cells [25]. Qin et al. [26] demonstrated that dihydroartemisinin increases Bim and Bak expressions and decreases Mcl-1 expression in human hepatocellular carcinoma cells thereby activates the intrinsic pathway of apoptosis. Other study revealed that dihydroartemisinin induces apoptosis in human leukemia cells in vitro and exhibits an anti-leukemic activity in vivo through a process that involves MEK/ERK inactivation, Mcl-1 down-regulation, culminating in cytochrome c release and caspase activation [27]. In another study, the effect of the combination of resveratrol and artemisinin on HepG2 and HeLa cells was investigated. Results showed that resveratrol and artemisinin inhibits the growth and migration of HeLa and HepG2 cells. Moreover, the combination of two compounds exhibited the synergistic anti-tumor activity [28]. The results of above

researches are in agreement with our data and show that dihydroartemisinin can potentially sensitize the tumor cells to anticancer compound, such as ABT-737, through induction of apoptosis.

The intrinsic or mitochondrial pathway of cell death is induced by an array of stimuli such as chemotherapeutic agent, hypoxia, radiation and free radicals. The regulation of this pathway is under the control of the pro- and anti-apoptotic members of the Bcl-2 family proteins [29-31]. In apoptotic conditions, the activated pro-apoptotic members such as Bak and Bax are inserted into mitochondrial membrane and cause the mitochondrial outer membrane permeability (MOMP). Release of cytochrome c from the mitochondrial intermembrane space triggers a cascade of caspase activation that results in cell apoptosis. The anti-apoptotic members such as Mcl-1 and Bcl-2, when not sequestered by Bak and Bax, inhibit apoptosis [29-31]. P21 acts as a cell cycle and growth inhibitor in normal cells that is deregulated in numerous cancer cells [32]. The role of P21 in the mitochondrial pathway of apoptosis is not precisely known and depends on multiple factors, such as the type of cells and the interplay between anti- and pro-apoptotic proteins [33]. ABT-737 is small molecule inhibitor of Bcl-2, Bcl-Xl, and Bcl-w proteins, but its affinity to Mcl-1 is low. Reports show that overexpression of Mcl-1 is linked to the ABT-737 resistance in tumor cells, and suppression of Mcl-1 expression has been proposed as a desirable strategy to enhance ABT-737 sensitivity of the cancer cells [22, 12, 13]. Previous studies have shown that dihydroartemisinin suppresses the cell cycle arrest in various types of malignant cells [14, 16]. Moreover, dihydroartemisinin alters the expression of P21, Bax, Mcl-1 and Bcl-2 via various cell signaling pathways such as NF-kB, MAPK, PI3K and JAK/STAT, thereby activates the external and internal pathway of apoptosis [14-16]. The results of our study showed that dihydroartemisinin lowers the expression of Mcl-1 and Bcl-2, and enhances the expression of Bax and P21 in ALL cells. These changes were connected to the induction of cell death. Moreover, down-regulation of Mcl-1 increases ABT-737sensitivity of the ALL cells. These data propose that dihydroartemisinin not only activates the intrinsic pathway of apoptosis, but also can increase the sensitivity of the ALL cells to ABT-737 through changing the Mcl-1expression.

In conclusion, we showed that combination treatment with dihydroartemisinin and ABT-737 significantly lowers in the IC_{50} and synergistically inhibits the cell growth and survival of the MOLT-4 and MOLT-17 ALL cells. Dihydroartemisinin induces the intrinsic pathway of apoptosis in ALL cells by overexpression of Bax and P21, and down-regulation of Bcl-2. Up-regulation of Mcl-1 was observed after exposure of the cells with ABT-737. Moreover, dihydroartemisinin enhances the apoptotic effect of ABT-737 by suppressing the Mcl-1 expression.

Author Contribution Statement

Study concept and design: HK and YA; Acquisition of data: RN, MP, JA and MD; Analysis and interpretation of data: HK, YA and MD; Drafting of the manuscript: RN, MP, JA and MD; Critical revision of the manuscript for important intellectual content: HK, RN and YA; Funding recipients: HK and YA.

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Ethical approval

This research was ethically wise approved from Deputy of research and technology, Arak University of Medical Sciences, Arak, Iran [Number 4172].

Conflict of interest

The authors have no conflict of interest to declare

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