RESEARCH ARTICLE

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Dual Targeting of Anti-Apoptotic Proteins Enhances Chemosensitivity of the Acute Myeloid Leukemia Cells

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Abstract

Background: Acute myeloid leukemia (AML) is a type of blood cancer characterized by fast cellular proliferation. Myeloid cell leukemia-1 (Mcl-1) and survivin, as anti-apoptotic proteins, are involved in cancer growth and resistance to chemotherapy. The aim of this study was to examine the combination effect of Mcl-1 and survivin specific siRNAs on chemosensitivity of the human HL-60 AML cells. **Methods:** SiRNAs transfection was performed by using Lipofectamine™2000 reagent. The mRNA expression was analyzed by real-time quantitative PCR. The apoptosis analysis was measured by ELISA cell death assay. **Results:** siRNAs markedly suppressed mRNA expression levels of Mcl-1 and survivin in a time-dependent manner, resulting in reduction of leukemic cell proliferation and enhanced spontaneous cell death. Surprisingly, Mcl-1 siRNA and survivin siRNA synergistically enhanced the cell toxic effects of etoposide. Furthermore, down-regulation of Mcl-1 and survivin significantly enhanced the apoptotic effect of etoposide. **Conclusions:** Our investigation suggests that suppression of Mcl-1 and survivin by siRNA can effectually inhibit cell growth and overcome chemoresistance of AML cells. Therefore siRNAs may be an important adjuvant in chemotherapy for AML patients.

Keywords: Acute myeloid leukemia- Etoposide- Mcl-1- SiRNA- Survivin

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Introduction

Acute myeloid leukemia (AML) is a hematologic malignancy and the common form of acute leukemia in adults (Moeinafshar et al., 2021). Standard strategies for treatment of AML are stem cell transplantation and chemotherapy (Greiner et al., 2019).Due to the development of graft-versus-host-disease (GVHD) and occurrence of relapse in hematopoietic stem cell transplantation, the treatment of this disease is still controversial (van Besien, 2013). In addition, in spite of intensive chemotherapy, the majority of patients with AML does not attain complete remission (CR) or are expected to relapse. This is mainly, owing to the development of multi-drug resistance in tumor cells (de Necochea-Campion et al., 2016; Ignatz-Hoover et al., 2018).Thus, the establishment of new therapeutic modalities is necessary.

Deregulation of the apoptosis, or programmed cell death, machinery contributes to the development of neoplasia and subsequent chemoresistance (Balaji et al., 2021; Yu et al., 2019). As the majority of the chemotherapy agents mainly exert their anti-tumor effects by the activation of apoptosis, new anti-cancer approaches have focused on targeting the mediators of this pathway (García-Vázquez et al., 2018; Xu et al., 2017). The apoptosis is exactly regulated by variety of protein member's families, like the inhibitor of apoptosis (IAP) proteins family and the anti-apoptotic members of B-cell lymphoma-2 (Bcl-2) families (Rathore et al., 2017).

Myeloid cell leukaemia-1 (Mcl-1), a tightly regulated member of the anti-apoptotic Bcl-2 family of proteins, was initially detected in the human myeloid leukemia cell line, ML-1, during differentiation into monocytes/macrophages (Beekman et al., 2016). Mcl-1 is expressed in different tissue and cancer cells and has a critical role in the control of cell cycle and apoptosis. Moreover, some studies have demonstrated that Mcl-1 is necessary for the function of hematopoietic cells and survival of malignant hematopoietic cells (Schulze-Bergkamen et al., 2008). Survivin (BIRC5) is the smallest member of the IAP family of proteins that is involved in the regulation of cell mitosis, survival and apoptosis. Unlike other IAP

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members, survivin is expressed primarily in embryonic and fetal tissues as well as in virtually every human tumor, but not in normal tissues (Filipchiuk et al., 2020).

Previous studies have shown that overexpression of both survivin and Mcl-1 is correlated with outgrowth, survival and resistance to conventional cancer therapy in many cancers including AML (F Liu et al., 2021; Zareifar et al., 2018). Owing to this overexpression in tumor cells, their critical role in cell death and cell division, as well as their correlation with chemoresistance, survivin and Mcl-1 have been considered as an potential therapeutic target in malignancies (Hormi et al., 2020; Santarelli et al., 2018). Moreover, various reports have shown that down-regulation of these proteins with small interfering RNAs (siRNAs), antisense oligonucleotides (ASOs), ribozymes and dominant negative mutants, increased apoptosis and sensitized tumor cells to conventional chemotherapeutic agents (Akagi et al., 2013; Quinn et al., 2011; Ryan et al., 2009; Zaffaroni et al., 2005).

The aim of this study was to investigate the inhibitory effects of simultaneous suppression of survivin and Mcl-1 on leukemia cells. We used a gene-silencing strategy using a pairs of specific siRNAs specifically knocking down the both genes to evaluate their cooperative effect on cell growth and apoptosis. We also assessed the potency of these siRNAs to sensitize HL-60 human AML cell line to chemotherapeutic agent etoposide in vitro

Materials and Methods

Cell culture conditions

The HL-60 human AML cell line (Pasteur Institute, Tehran, Iran) were maintained in RPMI-1640 medium (Sigma-Aldrich, St. Louis, MO, USA) containing 15% heat-inactivated fetal bovine serum (FBS) (Sigma-Aldrich), 2 mM of glutamine, and 1% sodium pyruvate, 1% antibiotics (100 µg/ml streptomycin,100 IU/ml penicillin) (Sigma-Aldrich) in a 5% CO2 humidified incubator at 37°C. The cells were cultured with an initial concentration of 6×104 cells/ml, passaged 48-72 h later and used in the logarithmic growth phase in whole experiments.

Transfection of siRNAs

The negative control (NC), Mcl-1 and Survivin siGENOME siRNAs were bought from Dharmacon (Lafayette, CO, USA). Before transfection, the leukemic cells were cultured in FBS and antibiotics-free growth medium. SiRNA transfection (at a final concentration of 50 nM in all experiments) was performed by using Lipofectamine™2000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. In brief, siRNAs and lipofectamine (4 µl/ml of transfection medium) were diluted in Opti-MEM I medium (Invitrogen) separately and incubated for 15 min at ambient temperature. Next, the diluted solutions were mixed and incubated for another 20 min at room temperature. Subsequently, the mixtures were added to the culture medium. The treated cells with only lipofectamine were considered as a siRNA blank control. Following on the cells were incubated for 6 h at 37˚C and then complete growth medium containing FBS (final FBS concentration of 10%) was added, with cells being incubated under the same conditions.

Quantitative real time PCR (qRT-PCR)

Total RNA was isolated by AccuZolTM reagent (Bioneer, Daedeok-gu, Daejeon, Korea) according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized from 1 µg of purified total RNA by use of MMLV reverse transcriptase (Promega, Madison, WI, USA) and oligo-dT primer following the manufacturer's protocol (Promega, Madison, WI, USA). QRT-PCR was performed on a LightCycler 96 System (Roche Diagnostics GmbH, Mannheim, Germany) by use of SYBR Premix Ex Taq (Takara Bio, Otsu, Shiga, Japan). RT-PCR reactions were performed in a total volume of 20 µl containing of 1 µL cDNA, 12 µl of SYBR green reagent, 0.2 µM of each of the oligonucleotides primers, and 6 µl of nuclease-free distilled water. The primer sequences were as follows: forward, 5'-TAAGGACAAAACGGGACTGG-3', reverse, 5'-ACCAGCTCCTACTCCAGCAA-3', for Mcl- 1, forward, 5'-GGACCACCGCATCTCTACAT-3', reverse, 5'-CAACGCGAAAGGAAAGACAG-3', for survivin, and forward, 5'-TCCCTGGAGAAGAGCTACG-3', reverse, 5'-GTAGTTTCGTGGATGCCACA-3', for β–actin. The PCR conditions for survivin, Mcl-1 and β–actin were 95oC for 10 min then 45 cycles at 95oC for 20 sec and 60oC for 1 min. Relative quantification of gene expression was measured with the $2^{-(\Delta\Delta Ct)}$ method (Ashofteh et al., 2021; Shahverdi et al., 2020; Shahverdi et al., 2021), using β-actin as an internal control (housekeeping genes)..

MTT assay

The effects of Mcl-1 and survivin specific siRNAs on sensitivity of the leukemic cells to etoposide (Sigma-Aldrich) were evaluated by 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide (MTT) assay. The experiment was divided to twelve groups: etoposide blank control, siRNA blank control, combination blank control, etoposide , NC siRNA, Mcl-1 siRNA, survivin siRNA, Mcl-1 siRNA + survivin siRNA, NC siRNA + etoposide, Mcl-1 siRNA + etoposide, survivin siRNA + etoposide, Mcl-1 siRNA + survivin siRNA + etoposide. Briefly, cells were cultured at a concentration of 15×103 cells/well in 96-well cell culture plates. Six hours after transfection, the cells were exposed to various concentrations of etoposide (0.001, 0.2, 0.5, 1, 2 and 4 µM). Cells treated with only 1% DMSO (solvent of etoposide), were considered as etoposide blank control. Treatment with a mixture of 1% DMSO and transfection reagent was also served as a combination blank control. After 18 h of incubation, the cytotoxic effects of the treatments were determined using a cell proliferation MTT kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's recommendations. The absorbance (A) was measured using a multi-plate reader (Awareness Technology, Palm City, FL, USA) at 570 nm with a reference wavelength of 650 nm. The survival rate (SR) was quantified from the following formula: SR (%) = (A Treatment /A Control) ×100%. Half max inhibitory (IC50) values were calculated

using Prism 6.01 software (GraphPad Software Inc, San Diego, CA, USA).

Analysis of combined drug effects

The combination index (CI) analysis based on the principles described by Chou-Talalay (Alamdari-Palangi et al., 2020; Amri et al., 2019) was performed to explore the interaction between siRNAs and etoposide. The results obtained with the MTT assay was converted to Fraction affected (Fa; where Fa = 0 is 100% cell survival and Fa = 1 is 0% cell survival) and analyzed by CompuSyn 1.0 software (ComboSyn Inc., Paramus, NJ, USA). Synergistic, additive, and antagonistic effects are indicated by CI of < 1, =1 or >1, respectively.

Cell proliferation assay

The effects of siRNAs on cell growth were explored by the trypan blue exclusion assay. Leukemic cells (5×104 cells/well) were transfected with siRNAs in 6-well culture plates and then incubated at 37˚C for 24-120 h. At different time points, the cells were harvested and then stained with equal volume of 0.4% trypan blue (Merck KGaA, Darmstadt, Germany) for 2 min. The number of viable cells (N, unstained blue) was counted under an inverted microscope (Nikon Instrument Inc., Melville, NY, USA) and a hemocytometer. The percentage of viable cells was determined by the equation as follows: Cell viability (%) = ($N_{Treatment}$ /$N_{Control}$) × 100. Moreover, the viability of the blank control group was considered as 100%.

Apoptosis ELISA assays

Leukemic cells were cultivated at a density of 1×105 cells/well in 6-well plates and then treated with Mcl-1 and survivin specific siRNAs, NC siRNA and the IC50 dose of erlotinib, alone and in combinations, as described in the MTT assay section. Following 24 h of incubation, apoptosis was assessed using an ELISA Cell Death Detection Kit (Roche Diagnostics GmbH), which measures mononucleosomes and oligonucleosomes produced in apoptotic cells. The absorbance was measured using an ELISA plate reader (Awareness Technology, Palm City, FL, USA) at 405 nm.

Statistical analysis

Quantitative data were presented as mean ± standard deviation (SD) of at least three independent experiments. Differences between groups were determined by using analysis of variance (ANOVA) and Bonferroni's test. All data were analyzed by GraphPad Prism software. Values of P less than or equal to 0.05 were considered statistically significant.

Results

Mcl-1 and survivin mRNA expression down-regulated with siRNA

To assess the effect of siRNA on Mcl-1 and survivin mRNA expression, the cells were transfected with 50 nM of each siRNAs for 24 and 48. Subsequently, RT-qPCR was performed to measure the expression of Mcl-1 and survivin mRNA. As shown in Figure 1, transfection of HL-60 cells with siRNAs led to a marked time-dependent reduction in mRNA expression levels relative to the blank control ($p < 0.05$). Transfection of Mcl-1 siRNA reduced the Mcl-1 mRNA expression to 64.38% and 58.43%, after

Table 1. IC_{50} Values of the Etoposide Alone and in Combination with siRNAs in Leukemia Cells

Treatment	IC_{50}
Etoposide	16.06 ± 1.08
Etoposide + NC siRNA	15.12 ± 0.33
Etoposide + Mcl-1 siRNA	10.41 ± 1.08*
Etoposide + Survivin siRNA	9.23 ± 0.33*
Etoposide + Mcl-1 siRNA + Survivin siRNA	7.08 ± 1.08#

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 etoposide; #p<0.05 versus single transfection.

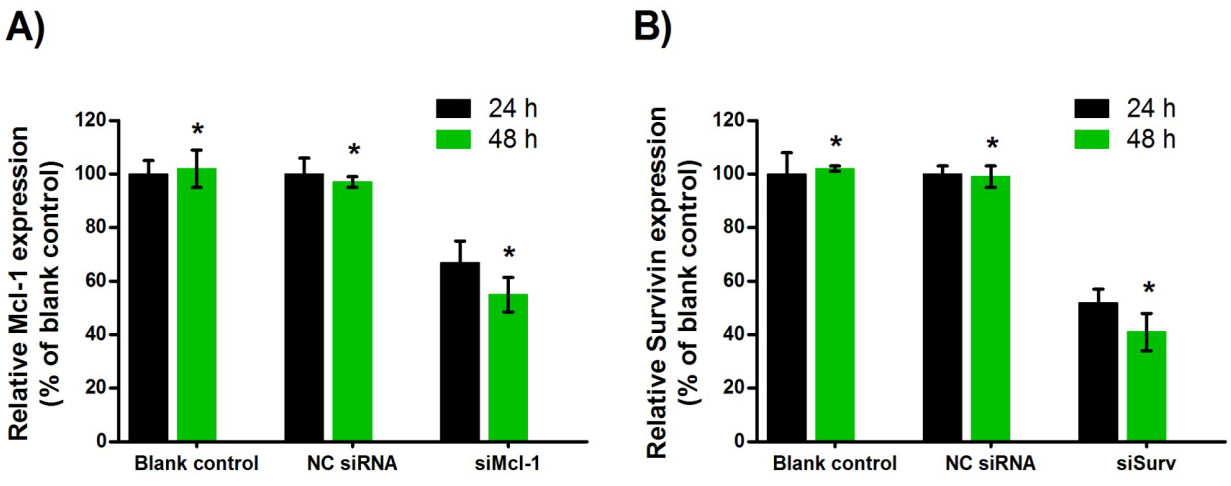

Figure 1. Mcl-1 and Survivin Expression Analysis in HL-60 Cells Treated with siRNA. The cells were transfected with NC siRNA, Mcl-1 siRNA and survivin siRNA for 24 and 48 h, and then relative Mcl-1 (A) and survivin (B) mRNA expression was measured by RT-qPCR. The results are expressed as mean±SD of three independent experiments. *p<0.05 versus blank control group or NC siRNA transfected cells

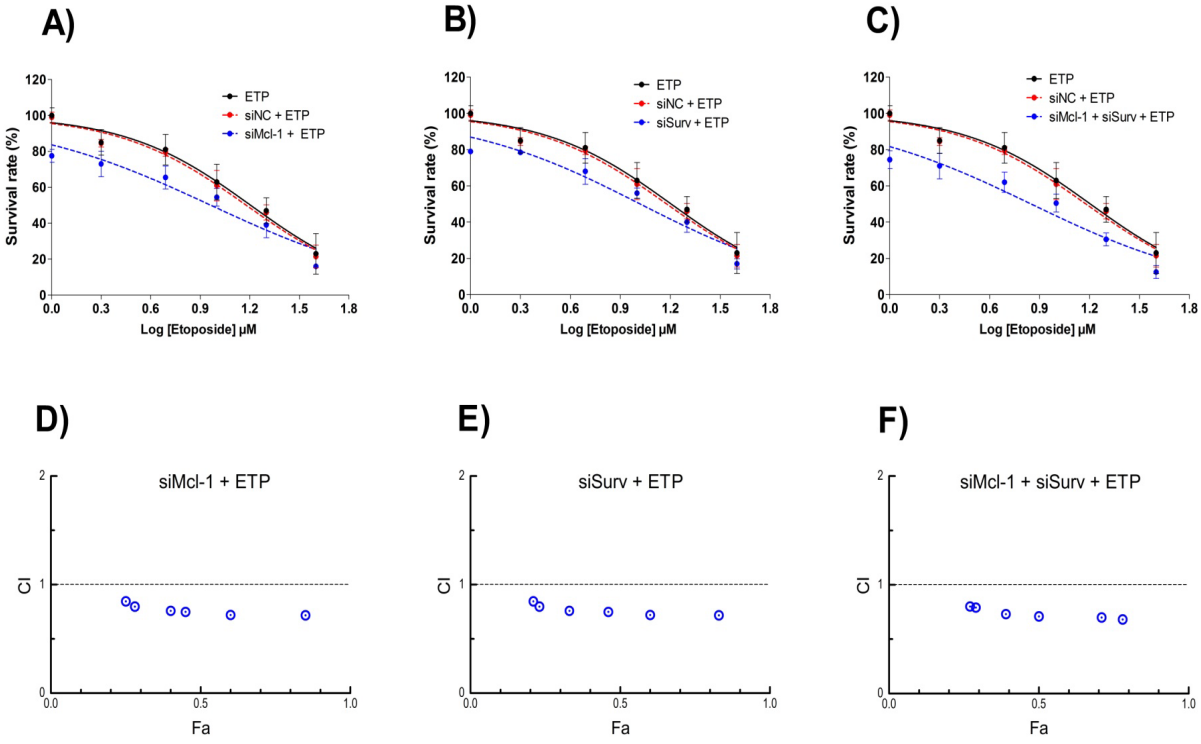

Figure 2. Effect of siRNAs in Combination with Etoposide on Cell Survival. The HL-60 cells were transfected with siRNA for 6 h and then exposed to etoposide at indicated concentrations. Twenty-four hours after transfection, the cell survival was measured using MTT assay. The cell survival curves were plotted by GraphPad software (A, B and C). Data are showed as mean ± SD of three experiments. The combination index (CI) values were calculated using the fractional affected (Fa) values of MTT assay and CalcuSyn software (D, E, F and G).

24 and 48, respectively ($p < 0.05$; Figure 1A). In addition, at the indicated time points, survivin siRNA significantly lowered the expression of survivin mRNA to 51.23% and 42.98%, respectively (Figure 1B). As expected, NC siRNA did not have a considerable effect on the Mcl-1 and survivin mRNA expressions levels compared to the blank control group ($p > 0.05$; Figure 1A, B).

Down-regulation of Mcl-1 and survivin sensitized HL-60 cells to etoposide

To investigate whether Mcl-1 siRNA and survivin

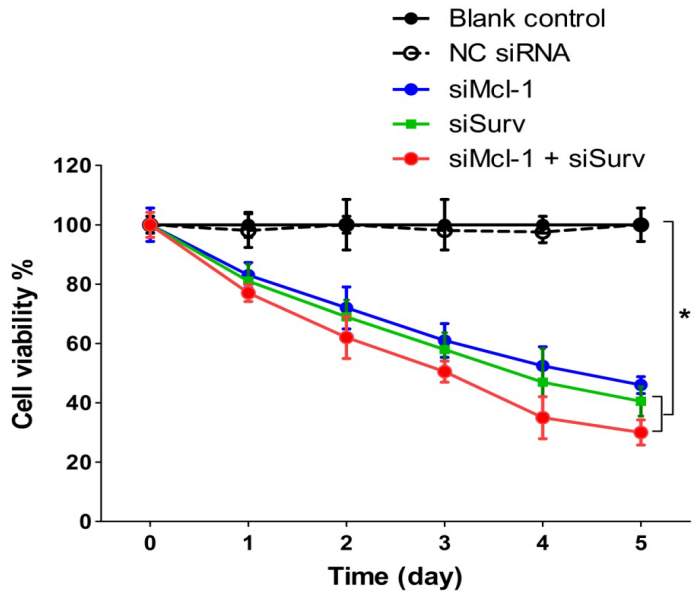

Figure 3. Proliferation Inhibition of HL-60 Leukemic Cells. The HL-60 cells were transfected with NC siRNA, Mcl-1 siRNA and survivin siRNA for 1-5 day, and the cell proliferation rate was measured using trypan blue assay at the end of each day. The data are represented as mean±SD of three experiments. *$p<0.05$ versus blank control or NC siRNA.

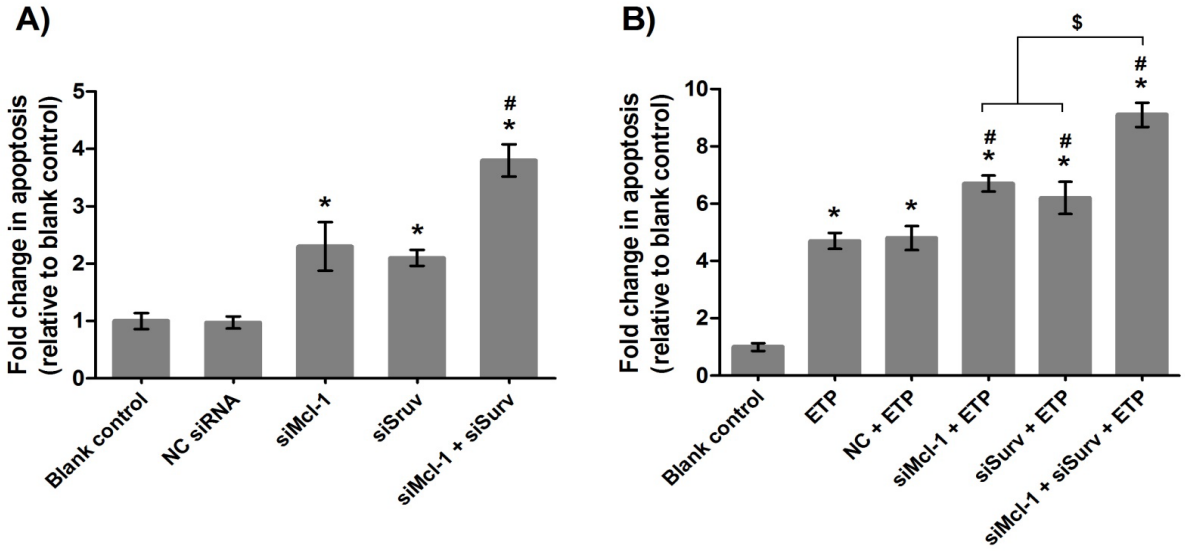

Figure 4. The Effect of siRNAs and Etoposide on Apoptosis of HL-60 Cells. Cells were treated with negative control (NC) siRNA, Mcl-1 siRNA, survivin siRNA and etoposide (IC50 doses of 24 h), alone and in combination. Next, apoptosis was assessed using ELISA cell death assay. The data are presented as mean ± SD (n=3). *p<0.05 relative to the control; #p<0.05 versus single transfection; $ p<0.05 relative to siRNA in combination with etoposode.

siRNA have the potential to sensitize HL-60 cells to etoposide, a combination treatment with siRNAs and etoposide were applied. As measured by MTT assay, monotherapy with etoposide caused a dose-dependent reduction of cell survival. Twenty-four hours after transfection of Mcl-1 siRNA and survivin siRNA, the cell survival rates reduced to 78.01% and 89.12%, respectively, relative to the blank control (Figure 2A, 2B; $p < 0.05$). Furthermore, compared with siRNAs or etoposide alone, combination treatment further decreased the cell survival of the HL-60 cells (Figures 2C; $p < 0.05$). The IC50 value of etoposide alone was 16.06 μM after 24 h (Table 1).We found that single transfection of Mcl-1 siRNA markedly reduced the IC50 value of etoposide to 9.24 μM at indicated time point. Moreover, transfection of survivin siRNA significantly lowered the IC50 of etoposide to 10.48 μM during the same time (Table 1). However, the combination of Mcl-1 siRNA and survivin siRNA further reduced the IC50 of etoposide to 7.08 μM (Table 1; $p < 0.05$; relative to the single transfection). The effect of negative control siRNA on the sensitivity of the cells to etoposide was minimal compared with etoposide alone ($p > 0.05$; Table 1)

Combination of Mcl-1 siRNA and survivin siRNA with etoposide synergistically inhibits survival of HL-60 cells

To assess whether the effects of siRNAs and etoposide on cell survival are responsible for their synergistic interaction, we determined the CI analysis based on the non-constant method of Chou–Talalay using CompuSyn software. The CI–Fa plots showed that the combination index of Mcl-1 siRNA (50 nM) or survivin siRNA (50 nM) with etoposide (2–64 μM) on HL-60 cells were synergistic interaction (CI < 1) in all of the combinations. Our findings demonstrated that strongest synergistic effects of 24 h were obtained at 8 μM etoposide in combination with Mcl-1 siRNA (CI = 0.78), survivin siRNA (CI = 0.80), and siRNAs cotransfection (CI = 0.70), with Fa levels of 0.41, 0.34, and 0.45, respectively (Figure 2D, 2E, 2F).

Mcl-1 and survivin siRNAs inhibited the proliferation of the HL-60 leukemia cells

As up-regulation of both Mcl-1 and survivin is correlated with growth and survival of leukemia cells, we therefore sought to examine whether down-regulation of these genes could suppressed the proliferation of the leukemia cells. Results demonstrated that compared with blank control group, specific siRNAs significantly decreased cell viability in a time-dependent way (Figure 3). At 24 h post-transfection of Mcl-1 siRNA and survivin siRNA, the cell viability reduced to 83.68% and 81.44%, respectively, and dropped to 43.38% and 40.63% on day 5. Moreover, simultaneous transfection both of siRNAs further decreased the cell proliferation rate, and cell viability dropped to 78.18% and 32.91% on days 1 and 5, respectively ($p < 0.05$, relative to single siRNA). In addition, no significant changed in cell proliferation were observed between NC siRNA and blank control group (Figure 3).

Combining Mcl-1 siRNA and survivin siRNA with etoposide led to insignificant enhancement of apoptosis in HL-60 cells

We then demonstrated that the sensitizing effect of specific siRNAs observed in MTT assay was correlated with enhancement of apoptosis. We used an ELISA-based cell death detection system to evaluate apoptosis. As shown in Figure 4A, 4B, 24 h treatment of the cells with each of siRNAs or etoposide alone resulted in notable enhancement of apoptosis compare to the blank control group ($p < 0.05$). Cotransfection of Mcl-1 siRNA and survivin siRNA triggered a massive apoptosis (Figure 4B).

Moreover, the combination of both siRNAs with etoposide further enhanced the amount of apoptosis relative to monotherapy (Figure 4B; $p < 0.05$). Interestingly, the cells cotransfected with Mcl-1 siRNA and survivin siRNA were more sensitive to etoposide-mediated apoptosis than cells transfected with either Mcl-1 siRNA or survivin siRNA (Figure 4B). However, the cells exposed to NC siRNA alone or in combination with etoposide showed no distinct differences in apoptosis compare to the blank control or etoposide-treated cells, respectively (Figure 4; $p > 0.05$). Together, these results indicate that Mcl-1 siRNA and survivin siRNA sensitize the HL-60 tumor cells to etoposide by induction of apoptosis.

Discussion

One of the important challenges in the treatment of AML patients is multi-drug resistance (de Necochea-Campion et al., 2016; Ignatz-Hoover et al., 2018). Therefore, the development of new treatment approaches is necessary. Several reports have indicated that up-regulation of the Mcl-1 and survivin is linked to the chemoresistance (F Liu et al., 2021; Zareifar et al., 2018). Other studies have demonstrated that suppression of Mcl-1 and survivin expression can sensitize tumor cells to anti-cancer agents (Akagi et al., 2013; Quinn et al., 2011; Ryan et al., 2009; Zaffaroni et al., 2005). In this study, we examined the combination effect of Mcl-1 and survivin specific siRNAs on sensitivity of the human HL-60 AML cells to etoposide.

The results of RT-qPCR revealed that transfection with Mcl-1and survivin siRNAs led to a significant decrease in the mRNA levels of corresponding genes during the 2-day period. These findings propose that specific siRNAs efficiently blocked the expression of the Mcl-1 and survivin. Cell proliferation analysis indicated that down-regulation of these genes could suppressed the proliferation of the AML cells. These results demonstrate that Mcl-1 and survivin play important role in proliferation of the HL-60 cells. The findings of MTT assay showed that pretreatment with siRNAs synergistically increased the cytotoxicity of etoposide in leukemia cells. These results suggest that inhibition of Mcl-1 and survivin could sensitize the AML cells to chemotherapy.

To further investigate the role of Mcl-1 and survivin silencing on chemoresistance of HL-60 tumor cells, we examined the effect of gene suppression on etoposide-mediated apoptosis. The results of the apoptosis showed that treatment with etoposide, alone, led to remarkable apoptosis in leukemia cells. Moreover, suppression of Mcl-1 and survivin expression increased the sensitivity of HL-60 cells to etoposide-induced apoptosis. Our findings are in agreement with the results of previous reports on a variety of cancer cells and, further confirm the role of Mcl-1 and survivin on chemoresistance of the tumor cells (Akagi et al., 2013; Song et al., 2008). For example, Liu et al. (X Liu et al., 2015) reported that Mcl-1 significantly up-regulated in triple-negative breast cancer cells and ectopic expression of miR-101 enhanced paclitaxel sensitivity of the MDA-MB-435 breast cancer cells. Similarly Li and colleagues (Li et al., 2016) showed that Mcl-1 is regulated by fork head box M1 (FoxM1) protein and inhibition of FoxM1 pathway can sensitize gastric cancer cells to cisplatin. In addition, Yoo et al. (Yoo et al., 2021) demonstrated that down-regulation of survivin by miRNA-138 enhanced caspase-dependent apoptosis of temozolomide in glioblastoma cells. The above-mentioned data suggest that the over-expression of Mcl-1 and survivin anti-apoptotic proteins is essential for the survival, proliferation and development of drug resistance in tumor cells. Therefore, suppression of these genes could stimulate apoptosis and sensitize AML cells to chemotherapy drugs.

Apoptosis can be occurred by one of two pathways, the intrinsic or mitochondrial pathway and the extrinsic or death receptor pathway. Both pathways induce cell death by activating caspases. Mcl-1 mostly suppresses the intrinsic pathway by neutralization of pro-apoptotic Bcl-2 family members. This situation inhibits the release of cytochrome c from mitochondria that is necessary for activation of caspase and next apoptotic events (Shahverdi et al., 2020; Shahverdi et al., 2021). Studies on melanoma cells have shown that over-expression of Mcl-1 inhibited the extrinsic pathway of apoptosis (Boisvert-Adamo et al., 2009). Survivin has been also showed to suppress apoptosis through the two apoptosis pathways by inhibition of caspases activities. However, the exact roles of Mcl-1 and survivin in regulation of cell death are still unclear (Church et al., 2012; Kelly et al., 2011).

Etoposide is a cell cytotoxic drug that induces programmed cell death by maintaining a covalent DNA topoisomerase II-DNA complex lead to DNA double strands to break. It was illustrated that etoposide induces apoptosis via both the intrinsic and extrinsic pathways by activation of different caspases (Montecucco et al., 2007). Our data demonstrated that decrease in Mcl-1 and survivin expression augments the cytotoxic effect of etoposide in HL-60 cells. So, we suggest that down-regulation of these genes by siRNA may increase the apoptotic effect of chemotherapeutic through caspase-dependent mechanisms. Therefore, more investigations on molecular mechanisms are needed.

SiRNA is a synthetic double-strand RNA duplex that transfected into target cells to represses gene expression through cleavage of the mRNA or translation inhibition. Because of the advantages of siRNA such as its specificity, efficacy, low cytotoxicity, and resistance to nuclease-induced degradation, it is largely used in cancer therapy studies (Karami,Baradaran,Esfehani, et al., 2014). In contrast, instability of double-stranded siRNA is one of the main defects of siRNA-based therapy which can be overcome by use of the constitutive siRNA-based vector systems (Karami,Baradaran,Esfahani, et al., 2014).

In conclusion, together, our investigation indicated that Mcl-1 and survivin proteins can play an important role in the survival, proliferation and resistance of HL-60 tumor cells to etoposide. Knockdown of both proteins using siRNA triggered significant cell death in leukemia cells in vitro and synergistically increased sensitivity of the AML cells to etoposide. Indeed, simultaneous suppression of Mcl-1 and survivin had a stronger effect on sensitizing HL-60 cell to etoposide than the independent suppression.

Our study also emphasizes potential of anti-apoptosis protein specific siRNAs for sensitization of HL-60 tumor cells to decrease side-effects of high-dose chemotherapy. So, simultaneous Mcl-1 and survivin targeting can be considered as a potent strategy in combination with chemotherapy for AML patients.

Author Contribution Statement

Study concept and design: BB and ES; Acquisition of data: HK, RN and ZA; Analysis and interpretation of data: HK, BB and ES; Drafting of the manuscript: RN, ZA and HK; Critical revision of the manuscript for important intellectual content: BB and ES; Funding recipients: HK, ES and BB.

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Ethical approval

This research was ethically wise approved from Deputy of research and technology, Tabriz University of Medical Sciences, Tabriz, Iran [Number 9032].

Conflict of interest

The authors have no conflict of interest to declare.

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