Türk Klinik Biyokimya Derg 2024; 22(2): 83-92

Araştırma Makalesi / *Research Article*

https://doi.org/10.56615/tkbd.2024.10

NF-kβ and MEK1/MEK2 expressions in parthenolide and cisplatin treated Leukemia cells

Partenolit ve sisplatin uygulanmış lösemi hücrelerinde NF-kβ ve MEK1/MEK2 ekspresyonları

Elif Kağa[1] **Tülay Köken**[2]

[1] Afyonkarahisar Sağlık Bilimleri Üniversitesi, Şuhut Sağlık Hizmetleri Meslek Yüksek Okulu, Tıbbi Laboratuvar Teknikleri Bölümü, Afyonkarahisar, Türkiye

[2] Afyonkarahisar Sağlık Bilimleri Üniversitesi, Tıp Fakültesi, Tıbbi Biyokimya Anabilim Dalı, Afyonkarahisar, Türkiye

Received / Başvuru Tarihi*:* 09 Temmuz 2024 *Accepted* / Kabul Tarihi*:* 13 Ağustos 2024

ABSTRACT

Aim: This study aimed to examine the effects of the NF-κβ inhibitor parthenolide, the anti-carcinogenic drug cisplatin and their combinations on the NF-κβ and MEK1/MEK2 expression levels and phosphorylation status in the human monocytic leukemia cell line (THP-1).

Materials and methods: The human monocytic leukemia cells (THP-1 cell line) were treated for 48h with various doses of parthenolide (5, 10, 20 µM), cisplatin (1, 3, 10 µM) and their combinations (1 µM Cis + 10 µM PTL and 3 µM Cis + 10 µM PTL). CD45, NF-κβ, MEK1/MEK2 expressions were analyzed by flow cytometry. The levels of NF-κβ, pNF-κβ, MEK1/MEK2, pMEK1/MEK2 proteins were detected by western blotting.

Results: The antiproliferative effect of parthenolide (10 µM) and cisplatin (1, 3 µM) was significantly increased with combination therapy (1 µM Cis + 10 µM PTL and 3 µM Cis + 10 µM PTL). NF-kβ expression level was significantly decreased at 5 µM PTL, 10 µM PTL groups and combination groups. Additionally, pNF-κβ phosphorylation decreased at the combination groups. MEK1/MEK2 expression was significantly increased at 5 µM PTL and 10 µM PTL groups.

Conclusion: The results show changes in expression of NF-kβ and MEK1/MEK2 proteins in leukemia cells treated with parthenolide and cisplatin combination. NF-kβ inhibitor parthenolide might be clinically useful, alone or in combination with chemotherapeutic agents, in particular with cisplatin for the treatment of hematological malignancies.

Key words: NF-kβ, MEK1/MEK2, Parthenolide, Leukemia

Yazışma adresi: Elif Kağa

Afyonkarahisar Sağlık Bilimleri Üniversitesi, Şuhut Sağlık Hizmetleri Meslek Yüksek Okulu, Tıbbi Laboratuvar Teknikleri Bölümü, Afyonkarahisar, Türkiye

e-posta: elif.kaga@afsu.edu.tr

Etik onay: Hücre hattı çalışması olması nedeniyle etik kurul onayı gerekmemektedir

ÖZET

Amaç: Bu çalışmada, insan monositik lösemi hücre hattında (THP-1 hücre hattı), pNF-κβ inhibitörü partenolit, anti-karsinojen ilaç sisplatin ve kombinasyonlarının pNF-κβ ve MEK1/MEK2 ekspresyon düzeylerine ve fosforilasyon durumlarına etkisinin incelenmesi amaçlanmıştır.

Gereç ve yöntem: İnsan monositik lösemi hücrelerine (THP-1 hücre hattı), 48 saat boyunca çeşitli dozlarda partenolit (5, 10, 20 µM), sisplatin (1, 3, 10 µM) ve kombinasyonları (1 µM Cis + 10 µM PTL ve 3 µM Cis + 10 µM PTL) uygulandı. CD45, NF-κβ, MEK1/MEK2 ekspresyonu akım sitometri ile analiz edildi. NF-κβ, pNF-κβ, MEK1/MEK2, pMEK1/MEK2 protein düzeyleri western blot yöntemiyle tespit edildi.

Bulgular: Partenolit (10 uM) ve sisplatinin (1, 3 uM) antiproliferatif etkisi, kombinasyon tedavisi (1 uM Cis + 10 uM PTL ve 3 uM Cis + 10 uM PTL) ile önemli derecede arttı. 5 µM PTL, 10 µM PTL gruplarında ve kombinasyon gruplarında NF-κβ ekspresyonu, anlamlı düzeyde azaldı. Ayrıca kombinasyon gruplarında pNF-κβ düzeyinde azalma görüldü. 5 µM PTL ve 10 µM PTL gruplarında, MEK1/MEK2 ekspresyonu önemli derecede arttı.

Sonuç: Sonuçlar, partenolit ve sisplatin kombinasyonu uygulanmış lösemi hücrelerinde NF-kβ ve MEK1/MEK2 proteinlerinin ekspresyonundaki değişiklikleri göstermektedir. NF-kβ inhibitörü partenolit, hematolojik malignitelerin tedavisi için tek başına veya özellikle sisplatin gibi kemoterapötik ajanlarla kombinasyon halinde klinik olarak faydalı olabilir.

Anahtar kelimeler: NF-kβ, MEK1/MEK2, Partenolit, Lösemi

INTRODUCTION

Acute myeloid leukemia (AML) is an aggressive hematologic cancer, characterized by disorders in hematopoiesis proliferation and differentiation (1, 2). The main acute leukemia treatment is chemotherapy includes pyrimidine nucleoside analogue, cytarabine or anthracyclines (daunorubicin or idarubicin). Despite treatment advances, the overall survival profile of patients with AML remains low and more effective treatments are required (3).

Abnormal activation of signal transduction pathways and growth factor receptors which include genetic defects, are involved in the pathogenesis of AML (4). Nuclear factor kappa β (NF-κβ) regulates important biological processes such as inflammation, cell proliferation and differentiation through signal mechanisms. In non-stimulated cells, NF-κβ is translocated into the cytoplasm by its inhibitor Iκβ. Various cellular events including cellular stress and infection lead to the phosphorylation of Iκβ proteins. Thus NF-κβ, released from Iκβ is activated and transported to the nucleus leading to the activation of target genes (5). Increased activation of NF-κβ has been shown in CD34+ stem cells from AML subtypes (6,7). NF-κβ signaling, which effects cell growth, differentiation, survival and resistance of leukemia cells, plays role in the pathogenesis of AML (8,9).

Mitogen-activated protein kinase kinase 1 (MEK1) and mitogen-activated protein kinase kinase 2 (MEK2) are protein kinases, involved in RAS-RAF-MEK-ERK signal transduction pathway that participate in the regulation of various cellular processes, including apoptosis, cell cycle progression, migration, differentiation and proliferation (10). Abnormal activity in this signaling pathway, which is involved in important cellular processes, causes uncontrolled tumor cell proliferation and survival. For this reason, it is shown as a therapeutic target for many types of cancer. Activated RAS-RAF-MEK-ERK pathway has been detected in approximately 30% of cancer cases (11).

Antimitotic agents, which affect the function of neoplastic cells by interfering with DNA, are commonly used in the treatment of AML. However, in the standard induction chemotherapy, most patinent achieve a

remission, but relapse is common due to population of chemotherapy-resistant leukemic stem cells after treatment. Standard chemotherapy often causes serious side effects in normal tissues (12).

Parthenolide is a sesquiterpene lactone isolated from the shoots of feverfew (Tanacetum parthenium). Parthenolide has been shown to have anticarcinogen and anti-inflammatory effects, making it a promising candidate for the treatment of cancer. Since its ability to target key signaling molecules and cancer stem-like cells, parthenolide has potential for the treatment of AML. It has shown promising results in inhibiting NF-κβ signaling, reducing pro-survival signaling pathways, inducing apoptosis, and targeting cancer stem-like cells in AML (13). It has been shown that combination therapy of anti-cancer drugs with parthenolide enhances treatment efficacy of AML in synergistic or an additive manner, compared to the standart therapy approaches thanks to its ability to target NF-κβ. Parthenolide significantly increased the sensitivity of human lung cancer to oxaliplatin, a cisplatin analogue, by inhibiting NF-κβ activation and inducing apoptosis (14).

In the study, parthenolide (PTL) and cisplatin (Cis) combination therapy was elavulated in human monocytic leukemia cells (THP-1 cell line) by the analysis of NF-κβ and MEK1/MEK2 expression and phosphorylation status. Cisplatin and parthenolide combinations were shown to be more successful than single-drug treatment according to NF-κβ, MEK1/MEK2 protein expression levels and pNF-κβ phosphorylation status.

MATERIALS AND METHODS

Reagents

Cell Counting Kit 8 (CCK-8) was purchased from Sigma-Aldrich. The leukemia cell line (THP-1) was obtained the American Type Culture Collection (ATCC, USA). RPMI-1640, fetal bovine serum (FBS) and Antibodies, CD45 APC, anti-MEK1/MEK2 APC, anti-NF-kappaB-p65 PE, anti-pMEK-1/pMEK-2 FITC, anti-pNF-κβ-p 65-PE, β-Actin were purchased from Invitrogen.

Cell culture

The human leukemic monocytic (THP-1) cell line cultured in RPMI (10% FBS, 1% P/S) at 37°C with 5% CO_2 environment.

Proliferation assay

THP-1 cells ($3x10^3$ cells/well) were seeded in 96-well and incubated for 24 h 37°C. Cells were treated with different concentrations of cisplatin (1, 3, 10 µM), Parthenolide (5, 10, 20 µM) and cisplatin+parthenolid combinations (1 μM Cis + 10 μM PTL and 3 μM Cis + 10 μM PTL) for 48 h. Cell survival was analysed according to the standard procedure of CCK-8 cell proliferation assay kit. 100 µL media containing 10 µL of CCK-8 solution was added to wells and the cells were further incubated for 4 h at 37°C. The absorbance were analysed at 450 nm using a plate reader (Thermo Scientific Mutilskan FC, USA). Cell viability was measured compared to OD value of control group. Proliferation analysis was performed at three replicates.

Flow cytometry

The THP-1 leukemia cells were treated with cisplatin (1, 3, 10 µM), parthenolide (5, 10, 20 µM) and cisplatin+parthenolid combinations (1 μM Cis + 10 μM PTL and 3 μM Cis + 10 μM PTL) for 48 h in cell culture conditions. The cells were washed with PBS, fixed and permabilized for 5 minutes. For the immunophenotypic analysis the samples were incubated with antibodies for 15 min in dark. Samples were analyzed by Backman Navios EX Flow Cytometer. A total of 10,000 events were recorded for each sample. Data analysis was performed with Kaluza Analysis Software (Beckman Coulter). After excluding cell debris and duplicates, the uniform cell population was plotted against FSC versus SSC. It was expressed as percentage cell number and arithmetic median.

Western blotting

THP-1 cells were taken with a cell scraper in RIPA buffer supplemented with protease inhibitors (Santa Cruz Bıotechnology, Inc. Europe) and lysed by incubating the samples on ice for 10 min. Protein concentration of samples were measured with BCA protein assay kit (Thermo, Rockford, IL, USA). Lysate protein (30 µg) was subjected to SDS-PAGE on 12% polyacrylamide gel and transferred onto nitrocellulose membrane (Bio-Rad, USA). The membrane was blocked with 5% non-fat dried milk in TBS with 0.1% Tween for 1 h. After the blocking, membrane was incubated with antibodies (Antibodies against NF-κβ-65, pNF-κβ-65, MEK1/MEK2, pMEK1/MEK2, β-Actin) at 4°C overnight. Mebrane was incubated with HRP-conjugated secondary antibody for 1h. Specific protein bands were dedected using an ECL western Blotting Substrate (Thermo Fisher Scientific Inc. USA). Bands was quantified by ImageJ Software.

Statistical analysis

Data analysis was performed using Ordinary One-way ANOVA test by the GraphPad Prism 8.0 software. Statistical significance was considered *P<0.05, **P<0.01 compared to the control group. # P<0.05 compared with the 3 µM Cis group.

RESULTS

Cytotoxic effect of parthenolide and cisplatin

According to results, treatment with 10 µM cisplatin significantly reduced the cells viability compared with control group. Moreover, a significant antiproliferative effect was determined in the 10 µM and 20 µM PTL groups compared to the control group. In the parthenolide and cisplatin combination groups (1 µM Cis + 10 µM PTL and 3 µM Cis + 10 µM PTL) significant inhibition of cell viability was observed compared with the control group. In 3 µM Cis + 10 µM PTL treated group significant inhibition of cell viability was observed compared with the 3 µM Cis group (Figure 1).

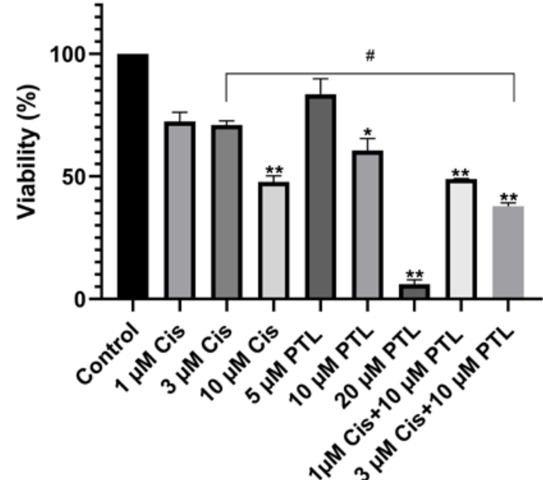

Figure 1. Inhibitory effects of different concentrations of cisplatin (1, 3, 10 µM), parthenolide (5, 10, 20 µM) and parthenolid + cisplatin combinations (1 µM Cis+10 µM PTL ve 3 µM Cis + 10 µM PTL) on THP-1 cell proliferation.

The results were presented as the mean ± SD of triplicate experiments. *P<0.05, **P<0.01 compared with control group. # P<0.05 compared with the 3 µM Cis group.

Şekil 1. THP- 1 hücre çoğalması üzerine farklı konsantrasyonlarda sisplatin (1, 3, 10 µM), partenolid (5, 10, 20 µM) ve partenolid + sisplatin kombinasyonlarının (1 µM Cis+10 µM PTL ve 3 µM Cis + 10 µM PTL) etkileri.

Sonuçlar üçlü deneylerin ortalama ± SD'si olarak sunuldu. *P<0.05, **P<0.01, kontrol grubuyla karşılaştırıldığında. # P<0,05, 3 µM Cis grubuyla karşılaştırıldığında.

Flow cytometry analysis

THP-1 cells were treated with 5 µM and 10 µM parthenolide for 48 hours in culture conditions. CD45, NF-κβ, MEK1/MEK2 expression was analysed in cell populations of parthenolide and control groups. In the control group, the size and granulation morphology of the cells were evaluated with SS (side scatter) and FS (forward scatter) analysis. Dead cells and debris were excluded from the analysis. In the control group, 97% of the analyzed cells was determined as CD45 positive cell population. In the 5 µM and 10 µM PTL groups, respectively 83% and 86% of cell population was determined as CD45 positive cell population (Figure 2).

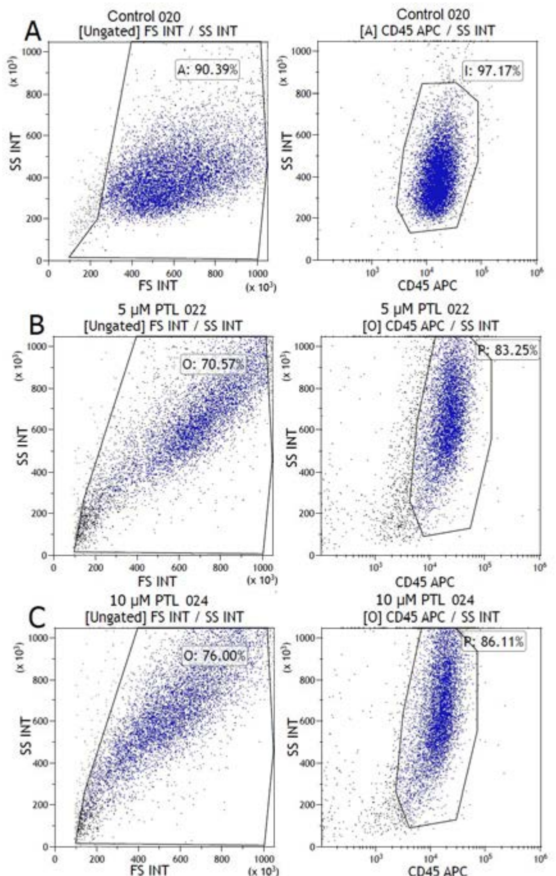

Figure 2. Flow cytometry analysis of THP-1 cells.

Plot is assigned FS vs SS to the population of all the cells. CD45 vs SS gates show the population of cells with positive CD45 and minimal side scatter.

A. Control cells. B. 5 μM PTL treated cells. C. 10 μM PTL treated cells. SS, side scatter, FS, forward scatter.

Şekil 2. THP-1 hücrelerinin akım sitometri analizi.

Temada, tüm hücrelerin popülasyonu FS ve SS olarak atanır. CD45 ve SS kapıları, pozitif CD45 ve minimum yan saçılıma sahip hücre popülasyonunu gösterir.

A. Kontrol hücreleri. B. 5 μM PTL ile işlemiş hücreler. C. 10 μM PTL ile işlenmiş hücreler. SS, yan saçılım, FS, ileri saçılım.

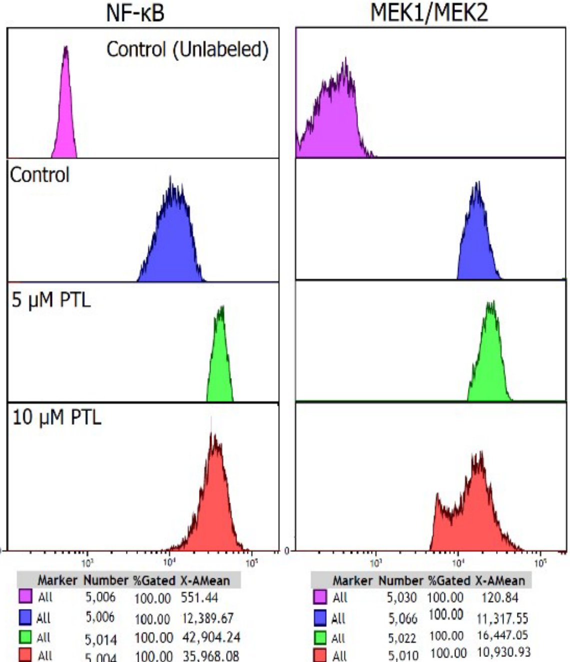

Figure 3. Flow cytometry analysis of NF-κβ and MEK1/MEK2 expression in THP-1 cell lines.

Cells were labeled by anti- NF-κβ -FITC, anti-MEK1/MEK2-APC.

Şekil 3: THP-1 hücre hatlarında NF-κβ ve MEK1/MEK2 ekspresyonunun akım sitometri analizi.

Hücreler anti-NF-κβ-FITC, anti-MEK1/MEK2-APC ile işaretlendi.

Figure 3 showed the effect of parthenolide on NF-κβ and MEK1/MEK2 expression in THP-1 monocytic leukemia cells. According to mean fluorescence intensity values, NF-κβ and MEK1/MEK2 were identified as positive in control group. In 5 μM and 10 μM parthenolide groups, there was an approximately 3-fold increase at NF-κβ expression compared to the control group and there was no significant change in MEK1/MEK2 expression.

NF-κβ, MEK1/MEK2 expression and phosphorylation status

According to Western blot results, NF-κβ expression was significantly decreased in 5 μM, 10 μM PTL groups and combination groups (1 μM Cis + 10 μM PTL and 3 μM Cis + 10 μM PTL) compared to the control group. Additionally, pNF-κβ phosphorylation levels in the combination groups decreased compared to the control group (Figure 4A).

MEK1/MEK2 expression and phosphorylation status were analysed by western blot. MEK1/MEK2 expression was significantly increased in 5 μM and 10 μM PTL groups compared to the control group. In the 10 μM Cis group, p-MEK1/MEK2 levels decreased compared to the control, and there was no change in p-MEK1/MEK2 expression in the combination groups (1 μM Cis + 10 μM PTL and 3 μM Cis + 10 μM PTL (Figure 4B).

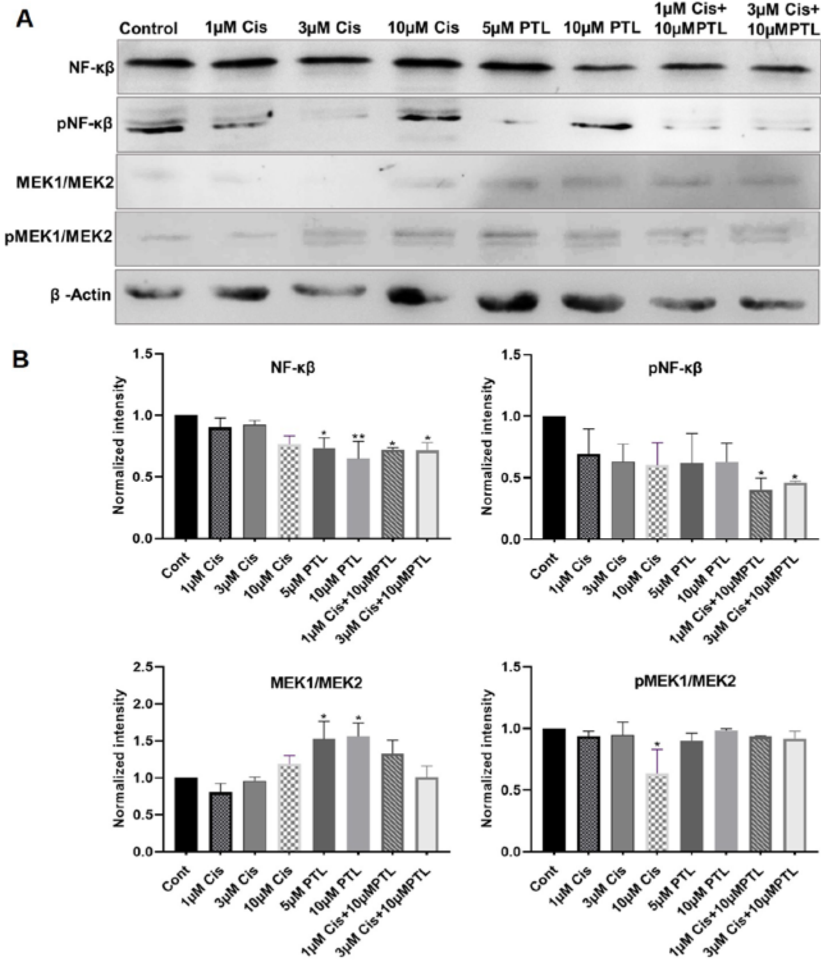

Figure 4. A) Western blot results show protein expression of NF-κβ-65, phospho-NF-κβ-p65, MEK1/2, phospho-MEK1/2 after treatment with cisplatin, parthenolide and its combinations in various doses. B) Densitometric analysis of the expression of NF-κβ, phospho-NF-κβ p65, MEK1/2, phospho-MEK1/MEK2, (each group, n=3).

The results were presented as the mean ± SD of triplicate experiments. *P<0.05, **P<0.01 compared with control group.

Şekil 4: A) Western blot sonuçları, çeşitli dozlarda sisplatin, partenolid ve bunların kombinasyonları ile tedaviden sonra NF-κβ-65, fosfo-NF-κβ-p65, MEK1/2, fosfo-MEK1/2'nin protein ekspresyonunu gösterir. **B**) NF-κβ, fosfo-NF-κβ p65, MEK1/2, fosfo-MEK1/MEK2 ekspresyonlarının dansitometrik analizi (her grup, n=3).

Sonuçlar üçlü deneylerin ortalama ± SS'sı olarak sunuldu. Kontrol grubuyla karşılaştırıldığında *P<0.05, **P<0.01.

DISCUSSION

In the study, the antiproliferative effect of cisplatin, parthenolide and its combination groups were examined in the human monocytic leukemia cell line (THP-1), and NF-κβ and MEK1/MEK2 expression and phosphorylation status were evaluated.

According to proliferation data, a significant antiproliferative effect was observed in the groups treated with 10 μM Cis, 10 μM PTL and 20 μM PTL in the THP-1 monocytic leukemia cell line. The anticarcinogenic potential of parthenolide has been demonstrated by cytotoxicity test with various cancer cell lines such as human lung carcinoma (A549), human medulloblastoma (TE671) and human colon adenocarcinoma (HT-29) (15). In the study, antiproliferatif effect of parthenolide and cisplatin combinations was analysed in THP-1 cells. According to the results, more cytotoxic

effects were observed in the cisplatin and parthenolide combination groups (1 μM Cis + 10 μM PTL and 3 μM Cis + 10 μM PTL) than the 10 μM PTL group and 1 μM cisplatin and 3 μM cisplatin groups. In the literature, it has been shown that parthenolide and cisplatin combinations showed cytotoxic effects depending on time and concentration in the cisplatin-resistant human gastric cancer cell line and that there is a synergistic effect between parthenolide and cisplatin (16).

In our study, the size and granulation morphology of the cells were evaluated by SS and FS analysis using flow cytometry and CD45 positive cells were gated in the examined cell line. Since normal leukocytes and hematopoietic precursors show typical SSC vs CD45 profile, characteristic phenotype is usually defined by SSC vs CD45 gate in flow cytometry AML immunophenotyping (17). Therefore, in the evaluation of NF-κβ and MEK1/MEK2 expression, the cell population included in the SSC vs CD45 gate was selected.

In addition, PLT was applied to THP-1 cells (5 μM and 10 μM) and changes in the expression level developed against the drug were analyzed by flow cytometry. An approximately 3-fold increase was detected in NF-κβ expression compared to the control group and no significant change was observed in MEK1/MEK2 expression. In the expression analysis performed by western blot, NF-κβ expression was significantly decreased in 5 μM and 10 μM PTL groups compared to the control group. MEK1/MEK2 expression was significantly increased in 5 μM and 10 μM PTL groups compared to the control group. Fixation step is applied before fluorescent labeling for internal protein analysis in flow cytometry. It has been reported that protein-protein interactions and changes in protein structures may occur as a result of fixation, which may cause signal loss at the expression level. In western blot method, since proteins are extracted from whole lysate, primary antibody access to proteins is higher (18). It is thought that the differences in NF-κβ and MEK1/MEK2 expression values obtained by western blot and flow cytometry occur due to these reasons.

The inhibition of NF-κβ has emerged as a promising therapeutic strategy for targeting various diseases, including cancer. NF-κβ can be used not only as a prognostic marker but also as a potential biomarker for targeted therapy of leukemia cells. Researchers have been able to induce apoptosis in cancer cells and suppress inflammatory responses by blocking the activity of NF-κβ, the transcription factor responsible for promoting cell survival and inflammation (19). Baumgartner et al. reported that NF-κβ activity was high in CD34+ and CD34- blasts in both new and recurrent AML patients (8). It has been reported that more than 50% of AML patient showed high level of NF-κβ activity and it is associated with more aggressive progression (20).

In the study, NF-κβ expression was significantly decreased in 5 μM, 10 μM PTL groups according to western blot results. Morever NF-κβ expression and phosphorylation in leukemic cells treated with cisplatin and parthenolide combinations (5 μM PTL+1 μM Cis and 5 μM PTL + 3 μM Cis) were significantly decreased compared to the control group. These results correlate with the data we obtained in the toxicity test. Increased NF-κβ expressions are considered a poor prognostic factor in many types of cancer. Parthenolide causes NF-κβ inhibition in leukemia stem cells and disrupts the redox balance. Therefore, the significant toxic effect of parthenolide on AML cells may be related to NF-κβ inhibition-induced apoptosis and increased ROS (21). Parthenolide significantly increased the sensitivity of human lung cancer cells to oxaliplatin, a cisplatin analogue, by inhibiting NF-κβ activation and inducing apoptosis (14). A similar study showed that parthenolide and cisplatin combination

increased with the activation of apoptosis in rat and human cell lines (22).

It has been suggested that there are interactions between the Raf/MEK/MAP kinase pathway and apoptosis regulating proteins in lymphocytic BAF/3 cells, since activated MEK-1 expression suppressed apoptosis (23). Additionally, the antiapoptotic effect of abnormal MEK-1 activity on hematopoietic cells (24, 25, 26) and increase in MEK activity in various leukemias, including ALL and CML (27) have been demonstrated.

In the study, MEK1/MEK2 expressions and phosphorylation statuses in leukemia cells were examined and the biological response to parthenolide and cisplatin and their combinations was evaluated. According to our flow cytometry results, MEK1/MEK2 expressions was positive in control THP-1 leukemia cells as similar studies in the literature. According to western blot analysis, MEK1/MEK2 expression was significantly increase in the 5 μM and 10 μM PTL groups. In a study conducted to investigate the effect of parthenolide as a B-Raf inhibitor, a decrease in the B-Raf expression and the phosphorylation of MEK and ERK was observed in GLC-82 cells treated with 20 µM parthenolide for 48 hours while the total protein level of MEK and Erk didn't change. To show whether parthenolide effect on B-Raf and c-Myc, siRNA interference was taken. According to siRNA interference results, parthenolide was shown to specifically target B-Raf and inhibit MAPK/Erk pathway signaling (28).

According to our data, parthenolide suppresses cell proliferation through NF-κβ inhibition, but caused an increase in MEK1/MEK2 expression. In parthenolide and cisplatin combination groups, MEK1/MEK2 expression decreased along with NF-κβ expression, and the antiproliferative effect increased. The decrease in MEK1/MEK2 expression shows the therapeutic effect of combination groups on leukemia cells. In a study effective leukemia treatment has been demonstrated through inhibition of MEK1/MEK2 signaling (29). Trametinib and cobimetinib are MEK inhibitors for metastatic melanoma with BRAF V600E mutation and have also been suggested to be effective in the treatment of acute myeloid leukemia (30).

Signal transduction pathways mediating cell proliferation, survival and apoptosis are important tools to examine more effective strategies in cancer treatment. In the present study, we demonstrated that parthenolid and cisplatin combinations inhibited leukemia cell prolyferation compared to single drugs at the same doses and reducing NF-κβ and MEK1/MEK2 expression, as well as reducing NF-κβ phosphorylation status. Combination therapy of chemoteray drugs with a NF- κβ or MEK1/MEK2 inhibitor would be a beneficial strategy to highger treatment efficiency and lower toxic side effects of chemothrapy drug and ultimately AML.

Conflict of interest: Authors have no conflicts of interest to declare.

Ethics committee approval

Commercially purchased cell line was used in the study. Therefore, it does not require any ethics committee approval.

Authors' contributions to the article

All authors contributed equally the main idea and hypothesis to this work. They both helped in preparing the manuscript.

Funding Statement

This study was supported by the Afyonkarahisar Health Sciences University, Scientific Research Projects Commission.

REFERENCES

1. Daver N., Schlenk RF., Russell NH., Levis MJ. Tareting FLT3 mutations in AML: Review of current knowledge and evidence. Leukemia. 2019;33:299–312.
2. Montalban-Bravo G., Garcia-Manero G. Novel drugs for older patients with acute myeloid leukemia. Leukemia 2015;29:760–769.
3. Francesco BA., Verzella D., Capece D., Vecchiotti D., Nolfi MDV., Flati ID. NF-kB: A Druggable Target in Acute Myeloid Leukemia. Cancers 2022;14:3557.
4. Frederick R., Appelbaum JM., Rowe JR., John ED. Acute Myeloid Leukemi. Department of Hematology and Bone Marrow Transplant, Rambam Medical Center, Haifa, 31096, 2001;Israel.
5. Shih RH., Wang CY., Yang CM. NF-kappaB Signaling Pathways in Neurological Inflammation: A Mini Review.Front Mol Neurosci. 2015;8:77.
6. Guzman ML., Neering SJ., Upchurch D., Grimes B., Howard DS., Rizzieri DA., Luger SM., Jordan CT. Nuclear Factor-KappaB is Constitutively Activated in Primitive Human Acute Myelogenous Leukemia Cells. Blood. 2001;98:2301–2307.
7. Baumgartner B., Weber M., Quirling M., Fischer C., Page S., Adam M., Von Schilling C., Waterhouse C., Schmid C., Neumeier D., et al. Increased IkappaB Kinase Activity Is Associated with Activated NF-KappaB in Acute Myeloid Blasts. Leukemia. 2002;16:2062–2071.
8. Labbozzetta M., Notarbartolo M., Poma P. Can NF-κB Be Considered a Valid Drug Target in Neoplastic Diseases Our Point of View. International Journal of Molecular Sciences. 2020; 21(9):3070.
9. Bosman MCJ., Schuringa JJ., Vellenga E. Constitutive NF-KB Activation in AML: Causes and Treatment Strategies. Crit. Rev. Oncol./Hematol. 2016;98:35–44.
10. Roskoski R Jr. MEK1/2 dual-specificity protein kinases: structure and regulation. Biochem Biophys Res Commun. 2012;417(1):5-10.
11. Ohren JF., Chen H., Pavlovsky A., Whitehead C., Zhang E., Kuffa P., Yan C., McConnell P., Spessard C., Banotai C., Mueller WT., Delaney A., Omer C., Sebolt-Leopold J., Dudley DT., Leung IK., Flamme C., Warmus J., Kaufman M., Barrett S., Tecle H., Hasemann CA. Structures of human MAP kinase kinase 1 (MEK1) and MEK2 describe novel noncompetitive kinase inhibition. Nat Struct Mol Biol. 2004;11(12):1192-7.
12. Guzman ML., Rossi RM., Karnischky L., Li X., Peterson DR., Howard DS., Jordan CT. The sesquiterpene lactone parthenolide induces apoptosis of human acute myelogenous leukemia stem and progenitor cells. Blood. 2005;105(11): 4163-9.
13. Baranello MP., Bauer L., Jordan CT., Benoıt SW. Micelle Delivery of Parthenolide to Acute Myeloid Leukemia Cells. Cellular and Molecular Bioengineering. 2018; 8(3):455–470.
14. Fang LJ., Shao XT., Wang S., Lu GH., Xu T., Zhou JY. Sesquiterpene lactone parthenolide markedly enhances sensitivity of human A549 cells to low-dose oxaliplatin via inhibition of NF-kappaB activation and induction of apoptosis. Planta Med. 2010;76:258–264.
15. Parada-Turska J., Paduch R., Majdan M., Kandefer-Szersze□ M., Rzeski W. Antiproliferative activity of parthenolide against three human cancer cell lines and human umbilical vein endothelial cells. Pharmacol Rep. 2007;59(2):233-7.
16. Li H., Lu, H.; Lv, M.; Wang, Q.; Sun, Y. Parthenolide facilitates apoptosis and reverses drug-resistance of human gastric carcinoma cells by inhibiting the STAT3 signaling pathway. Oncol. Lett. 2018;15:3572–3579.
17. Lacombe F., Durrieu F., Briais A., Dumain P., Belloc F., Bascans E., Reiffers J., Boisseau MR., Bernard P. Flow cytometry CD45 gating for immunophenotyping of acute myeloid leukemia. Leukemia 1997;11:1878–1886.
18. He L., Fox MH. Comparison of flow cytometry and western blotting to measure Hsp70. Cytometry. 1996;25(3):280-6.
19. Xia L, Tan S, Zhou Y, et al. Role of the NF□B-signaling pathway in cancer. Onco Targets Ther. 2018;11:2063-2073.
20. Cilloni D., Martinelli G., Messa F., Baccarani M., Saglio G. Nuclear factor B as a target for new drug development in myeloid malignancies. Haematologica 2007; 92:1224–1229.
21. Zhang H., Fang H., Wang K. Reactive oxygen species in eradicating acute myeloid leukemic stem cells. Stem Cell Investig. 2014;1:13.
22. Zanotto-Filho A., Braganhol E., Schröder R., de Souza LH., Dalmolin RJ., Pasquali MA., Gelain DP., Battastini AM., Moreira JC NFκB inhibitors induce cell death in glioblastomas. Biochem. Pharmacol. 2011;81:412–424.
23. Cseh B., Doma E., Baccarini M. "RAF" neighborhood: Protein–protein interaction in the Raf/Mek/Erk pathway. FEBS Lett. 2014; 588(15): 2398–2406.
24. Blalock WL., Pearce M., Steelman LS., Franklin RA., McCarthy SA., Cherwinski H., McMahon M., McCubrey JA. A conditionally active form of MEK1 results in autocrine transformation of human and mouse hematopoietic cells. Oncogene 2000;19: 526–536.
25. Blalock WL., Moye PW., Chang F., Pearch M., Steelman LS., McMahon M., McCubrey JA. Combined effects of aberrant MEK1 activity and BCL2 overexpression on relieving the cytokinedependency of hematopoietic cells. Adv Enzyme Regl 2000;40: 305–337.
26. Blalock WL., Pearce M., Chang F., Lee JT., Pohnert S., Burrows C., Steelman LS., Franklin RA., McMahon M., McCubrey JA. Effects of inducible MEK1 activation on the cytokine dependency of lymphoid cells. Leukemia 2001;15: 794–807.

27. Jr JL., McCubrey J. The Raf/MEK/ERK signal transduction cascade as a target for chemotherapeutic intervention in leukemia. Leukemia 2002;16, 486–507.
28. Minting L., Hong B., Yanyan Y., Wenjing H., Guiping Z., Genshui Z.1, Sili T., Yun L., Lingling Z., Jinxiang M., Jianye Z. Parthenolide suppresses non-small cell lung cancer GLC-82 cells growth via B-Raf/MAPK/Erk pathway. Oncotarget 2017;8(14):23436-23447.
29. Steelman L., Franklin R., Abrams S. et al. Roles of the Ras/Raf/MEK/ERK pathway in leukemia therapy. Leukemia 2011;25:1080–1094.
30. Welsh SJ., Rizos H., Scolyer RA., Lon GV. Resistance to combination BRAF and MEK inhibition in metastatic melanoma: Where to next European Journal of Cancer 2016; 62:76-85.