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Research Article

The mechanism of apoptosis in human acute promyelocytic leukemia cells treated with sorafenib and lithium chloride

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Abstract

Objectives: This study was an investigation of the mechanisms of sorafenib (SOR) and lithium chloride (LiCl), which cause apoptosis, in the acute promyelocytic leukemia (APL) HL-60 cell line.

Methods: HL-60 cells were treated with 100 μM of SOR, LiCl, and a combination of the 2 drugs, and a control group was not treated. Cells were collected after a period of 24, 48, and 72 hours, and cell proliferation and the apoptotic index were assessed with a hemocytometer and flow cytometry analysis. The level of caspase-3, phospho-glycogen synthase kinase-3 beta (p-GSK-3β), phospho-protein kinase B (p-AKT), phospho-extracellular-signal-regulated kinase (p-ERK), p38, phospho-c-Jun (p-c-Jun), and phospho-inhibitor kappa B (p-IκBα) were analyzed using the enzyme-linked immunosorbent assay method. The effects of the drugs on cell ultrastructure were evaluated with a transmission electron microscope (TEM).

Results: Single and combination drug administration decreased cell proliferation and increased the apoptosis rate ($p<0.01$ for both). The increase in apoptosis in the SOR+LiCl group was greater than that of the SOR group ($p<0.01$); however, there was no significant increase compared with the LiCl group. While both drugs increased the caspase-3 level ($p<0.01$ for both), LiCl increased caspase-3 activity more than SOR. Although p-GSK-3β levels decreased in the SOR group ($p<0.01$), levels increased in the LiCl group ($p>0.05$). Combined drug administration decreased the level of p-AKT and p38 ($p<0.01$ for both); however, it did not significantly affect the level of p-ERK, p-IκBα, or p-c-Jun ($p>0.05$). TEM examination revealed severe lytic cytoplasmic damage and apoptotic morphology, an indication of apoptosis.

Conclusion: The results of this study demonstrated that in human APL cells treated with SOR and LiCl, increased apoptosis led to a decrease in tumor cells. This combination may become a preferred drug alternative for patients with APL and a mood disorder.

Keywords: Acute promyleocytic leukemia, apoptosis, cell signaling pathway, lithium chloride, sorafenib

In the last decade, considerable evidence has been presented that has helped to understand the molecular components that contribute to the pathogenesis of acute myeloblastic leukemia (AML) [1]. AML accounts for approximately 15% of childhood leukemia, which includes the acute promyelocytic leukemia (APL) subtype [2]. All-trans retinoic acid, anthracycline antibiotics, and arsenic trioxide (ATO) are effective agents used in the treatment of APL; however, other treatment options become important when drug resistance develops [3].

Apoptosis can be triggered by various signals that occur at the extracellular and cellular levels. Caspase-3 is a molecule involved in the death receptor-mediated apoptotic (extrinsic) pathway [4]. Although the pathways involved in the dif-

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ferentiation of blood cells are very complex, 2 pathways are particularly important: the Ras-mitogen-activated protein kinase (MAPK) and the Janus kinase-signal transducer and activation of transcription (JAK-STAT) [5, 6]. The Ras-MAPK pathway enables the transmission of cytokine signals in the proliferation and differentiation of hematopoietic cells. Among the many known Ras effectors, phosphoinositide 3-kinase, serine/threonine-kinase, and Raf are important. The shelf binds to the active form of Ras and travels from the cytoplasm to the cell membrane. In the presence of cytokine receptors, a member of the JAK-2 and src-kinase family activates MAPKs by allowing tyrosine phosphorylation of Raf. MAPK activates extracellular-signal-regulated kinase (ERK), ERK enters the nucleus and enables activation of target genes [5, 6]. In the JAK-STAT pathway, cytokine receptors activate tyrosine kinases. With tyrosine kinase activation, JAK molecules combine with cytokine receptors to phosphorylate STAT molecules. Phosphorylated STATs form dimers, which are directed to the nucleus and bind to specific DNA regions to enable the activation of genes related to differentiation [5, 7, 8].

Sorafenib (SOR), an oral multi-tyrosine kinase inhibitor, has been used in the treatment of liver and kidney cancer since 2005 [9], and was recently approved by the US Food and Drug Administration to treat thyroid cancer [10]. Vascular endothelial growth factor, c-kit, platelet-derived growth factor receptors, BRAF, and Fms-related tyrosine kinase 3 (FLT3) are the target molecules [1]. There are studies indicating that different types of cancer cells cause apoptosis via the Ras/Raf/mitogen-activated protein kinase (MEK)/ERK pathway [11-14]. Lithium is a drug that may be used in the long-term treatment of bipolar disorder (manic-depressive disease), and recent studies have reported antitumor properties via glycogen synthase kinase-3 beta (GSK-3β) inhibition [15].

To the best of our knowledge, this study was the first to examine the application of SOR and lithium chloride (LiCl) to human HL-60 APL cells in vitro and the effects on the proliferation and apoptosis of leukemia cells, with the ultimate goal of contributing to APL treatment strategies.

Materials and Methods

Cell cultures

An HL-60 human Acute Promyelocytic Leukemia cell line (CLL-240) from the American Type Culture Collection cell bank (Rockville, MD, USA) was used in the present study. The medium used for the HL-60 cells was Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 Ham (DMEM-F12), containing inactivated 10% fetal bovine serum, with 0.2 mM glutamine, 100 µg/mL streptomycin, and 100 IU/mL penicillin (Sigma-Aldrich Corp., St. Louis, MO, USA). Cells were grown in 25 cm^2 and 75 cm^2 flasks containing this medium and kept in an incubator maintained at 5% CO_2, 95% humidity, and 37°C, and routine passaging was performed twice a week.

Dose determination test

Adherent cells on the upper layer of the medium and flask surface were collected with a pipette and transferred to 15 mL centrifuge tubes. The cells were centrifuged at 1500 rpm for 5 minutes. In each well of 6-well culture dishes, 500.000 100% viable HL-60 cells were seeded in 5 mL of DMEM-F12. Fresh solutions of LiCl (L4408; Sigma-Aldrich Corp., St. Louis, MO, USA) and SOR (Bayer AG, Leverkusen, Germany) were prepared at concentrations of 1,10,100, and 200 µM and delivered in equal volumes of 100 µL. The addition was performed 3 times for each dose of the drugs. All of the groups were seeded for 24, 48, and 72 hours and incubated in a humid environment at 37°C in a 5% CO_2 air mixture.

After 24, 48, and 72 hours, the cells in the wells were collected separately and centrifuged. After the supernatant fractions were discarded, 1 mL of medium was suspended and a hemocytometer (Neubauer) was used to perform total cell counts.

After determining the inhibitory dose 50% (ID_{50}) value, a new experiment was performed for the 2 drugs to be analyzed. The ID_{50} value was 100 µM for both LiCl and SOR (GraphPad Prism 5; GraphPad Software Inc., San Diego, CA, USA).

The experimental groups were the Control Group, to which no additional material was added; the SOR-only Group, to which 100 µM SOR was added; the LiCl-only Group, to which 100 µM LiCl was added; and the LiCl+SOR Group, to which 100 each µM LiCl and SOR were added.

Proliferation experiment and determination of viability

At the end of every 24 hours, cells were harvested by centrifugation and counted using a hemocytometer (Neubauer). The viable cell count was determined using a light microscope (Olympus CX21; Olympus Corp., Tokyo, Japan) to examine a preparate derived from a 1/1 cell suspension prepared with 0.1% trypan blue.

Detection of apoptosis by flow cytometer

Cells were harvested at the end of each 24 hour-period (each group was run 3 times), counted with a counting chamber, and washed twice with cold phosphate buffered saline (PBS) containing 0.9% sodium chloride. The cells were then resuspended (1×10^6 cells in 1 mL binding solution) with binding buffer diluted x10 with distilled water. Next, 100 µL of this suspended form was transferred to another tube. (1×10^5 cells per 100 µL) and 5 µL of FITC Annexin V (BD Pharmingen, BD Biosciences, San Jose, CA, USA) was added, followed by 10 µL propidium iodide (PI). The cells were gently vortexed and incubated for 15 minutes at room temperature (25°C) in the dark before 400 µL of binding buffer was added to each tube. Fluorescence with annexin-V and PI was measured within 1 hour using flow cytometry. The results were evaluated with BD CellQuest (BD Biosciences, San Jose, CA, USA) and the WinMDI software of the cytometer (Windows Multiple Docu-

ment Interface for Flow Cytometry; J. Trotter), and the dead, viable, and apoptotic cell ratios were determined for each drug application.

Caspase-3, p-GSK-3β, p-ERK, p-AKT, p-p38, p-c-Jun, p-IκBα and p-STAT3 levels

The enzyme-linked immunosorbent assay (ELISA) method was used to analyze each signaling molecule: Caspase-3 (Colorimetric Caspase 3 Activity Assay Kit; Sigma-Aldrich Corp., St. Louis, MO, USA); phospho-glycogen synthase kinase-3 beta (p-GSK-3β) (Phospho-Ser9 EIA; Wuhan USCN Business Co., Ltd., Wuhan, China); and phosphor-extracellular-signal-regulated kinase (p-ERK), phospho-protein kinase B (p-AKT), p-p38, p-c-Jun, p-inhibitor kappa B (IκBα), and p-STAT3 (each Cell Signaling Technology, Inc., Danvers, MA, USA). The analysis was based on the principle of the solid-phase sandwich ELISA technique, which allows for the measurement of multiple protein levels. The experiment was repeated 3 times for each group.

Ultrastructure studies

Cells from the control and all experimental groups were collected at 72 hours. The medium was removed from the centrifuge tubes and discarded and the samples were washed with PBS followed by 1.5% glutaraldehyde and maintained at 30°C for 30 minutes. After washing 2 more times with PBS, centrifugation was performed, and supernatant was withdrawn and incubated in osmium tetraoxide for 1 hour at 4°C. After another wash in PBS for 10 minutes, the samples were kept in 1% uranyl acetate for 15 minutes. Incubation in 30% ethanol was performed for 10 minutes. Pre-filtered, fresh egg white was placed on the cells and centrifuged to keep the cells together. After whole egg whites were separated, the procedure was continued with 10 minutes in 50% ethanol, 10 minutes in 70% ethanol, 10 minutes in 2x 100% ethanol, 10 minutes in 2x pure propylene oxide, 1 hour in a propylene oxide/epon mixture prepared in 1/1 ratio, 1/3 ratio prepared propylene oxide/epon mixture for 1 hour, and pure epon for 1 hour to complete the embedding process. The blocks were then placed in the oven for 18 hours at 37°C, trimmed, and thin sections were cut and transferred to copper grids. Lead citrate and uranyl acetate were contrasted and examined with a JEOL-JEM 1011 (JEOL, Ltd., Tokyo, Japan) transmission electron microscope (TEM).

Statistical analysis

SPSS Statistics for Windows, Version 17.0 (SPSS, Inc., Chicago, IL, USA) software was used to analyze the data. The results were shown as mean±standard error mean. Differences between groups were calculated using Student's t-test. A probability value of $p<0.05$ was considered significant.

Results

Cell proliferation index

Cells in the control group, which had no drugs administered, grew exponentially from the beginning of the experiment to the end of the 72-hour period, as expected. All of the drug applications demonstrated a decrease in the number of cells at the conclusion of 72 hours ($p<0.01$). There was a statistically significant decrease in cell count only in the combined drug usage relative to SOR ($p<0.01$). The inhibition of the cell proliferation index was highest in the combination group, followed by the LiCl and SOR groups, respectively ($p<0.01$) There was no statistically significant difference in the number of cells compared to LiCl-only at the 24th, 48th, and 72nd hours ($p>0.05$) (Fig. 1).

Apoptotic index

The lowest apoptotic index was found in the control group: Each of the 3 drug applications demonstrated an increase when compared the control (for each $p<0.01$). The increase was highest in the combination group, followed by the SOR and LiCl groups, respectively ($p<0.01$). At 24 hours, the LiCl group showed no significant difference when compared with the combination group ($p>0.05$); however, there was a statistically significant increase in the rate of programmed cell death at 48 hours and 72 hours ($p<0.001$, $p<0.01$, respectively) (Fig. 2a, 2b).

Caspase-3 activity

All 3 treatments increased caspase-3 activity ($p<0.01$). The combination treatment induced the greatest increase, followed by the LiCl and SOR groups, respectively ($p<0.01$) (Fig. 3a).

Level of phosphorylated GSK-3β

SOR induced a decrease in p-GSK-3β at 24 hours and 72 hours ($p<0.01$). LiCl increased the p-GSK-3β level significantly at 24

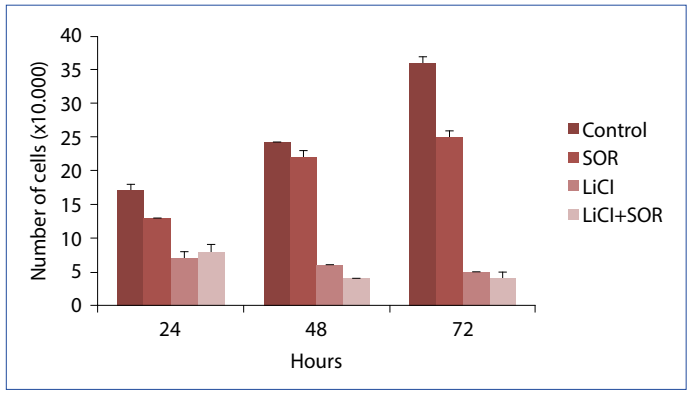

Figure 1. Time-dependent cell count in all of the study groups Mean±SEM.
LiCl: Lithium chloride; SOR: Sorafenib.

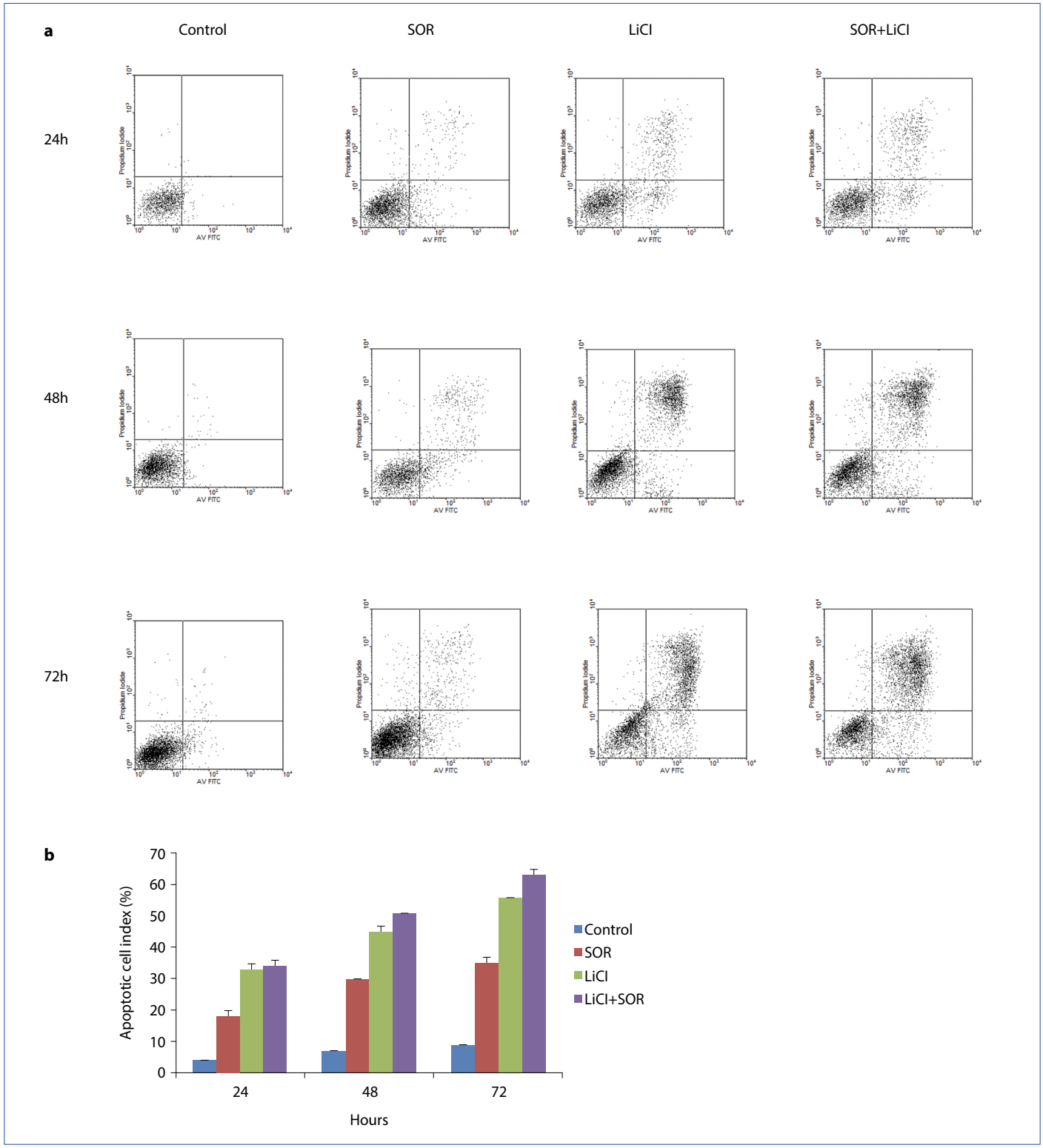

Figure 2. (a) Histogram showing the distribution of apoptotic cells in samples treated with sorafenib (SOR) and lithium chloride (LiCl) at 24, 48, and 72 hours determined using avidin V fluorescein isothiocynate. (b) Graph illustrating the mean, early, and late apoptotic cell ratios±SEM values at 24, 48, and 72 hours in each group.

hours ($p<0.05$), but there was no significant difference at 72 hours ($p>0.05$). At 24 hours, p-GSK-3β was lower in the combination group compared with the control group ($p<0.01$). When compared with the SOR group, the combination group demonstrated a higher level of p-GSK-3β ($p<0.05$), which was presumably provided by LiCl (Fig. 3b).

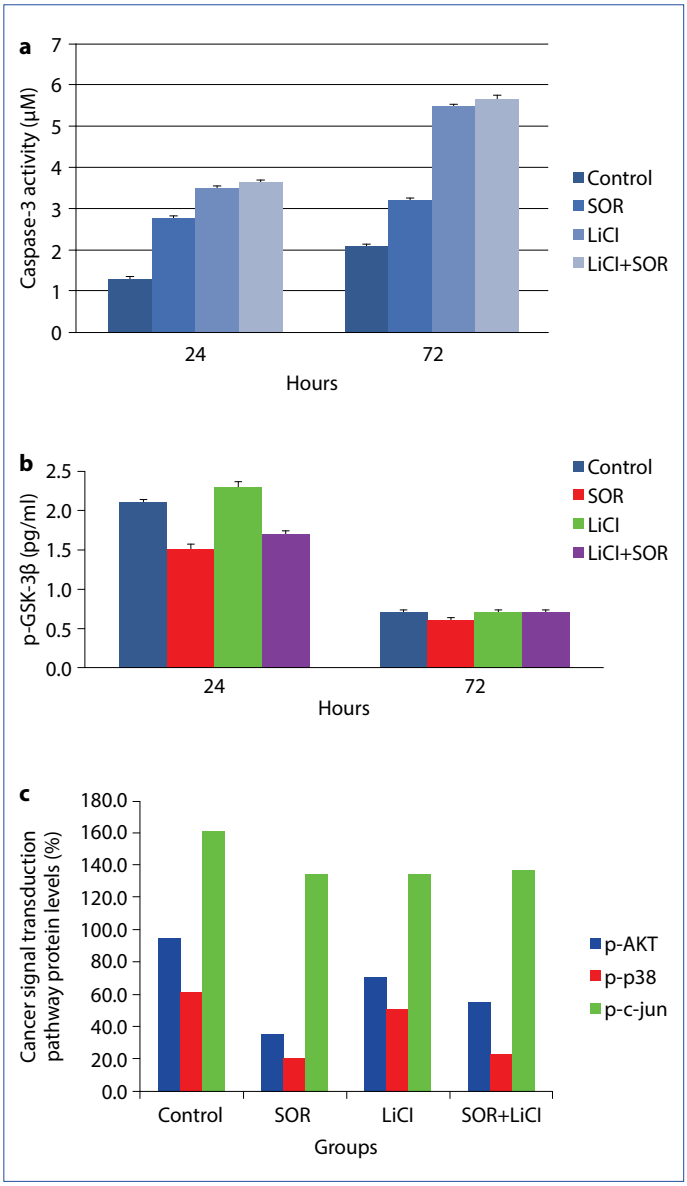

Figure 3. (a) Caspase-3 activity; mean (μM)±SEM. (b) Phospho-glycogen synthase kinase 3-beta levels; mean (pg/mL)±SEM. (c) Cancer signal transduction pathway protein levels; mean (%)±SEM.

LiCl: Lithium chloride; p-AKT: Phospho-protein kinase B; p-c-Jun: Phospho c-Jun; p-GSK-3 beta: Phospho-glycogen synthase kinase 3-beta; p-p38: Phospho p38; SOR: Sorafenib.

Level of proteins of signal transduction pathway

SOR induced the greatest decrease in p-AKT, p-p38, and p-c-Jun ($p<0.01$). The combination group demonstrated the next greatest decrease in p-AKT and p-p38 levels ($p<0.05$).

The effect of LiCl on p-c-Jun levels was similar to that of SOR when compared with the control group ($p<0.01$) (Fig. 3c). The effect of the 2 drugs on p-ERK and p-IκBα was compared with the control group and between them, and no statistically significant difference was observed ($p>0.05$).

The levels of p-STAT3 were undetectable; therefore, evaluation could not be performed (not shown in the figures).

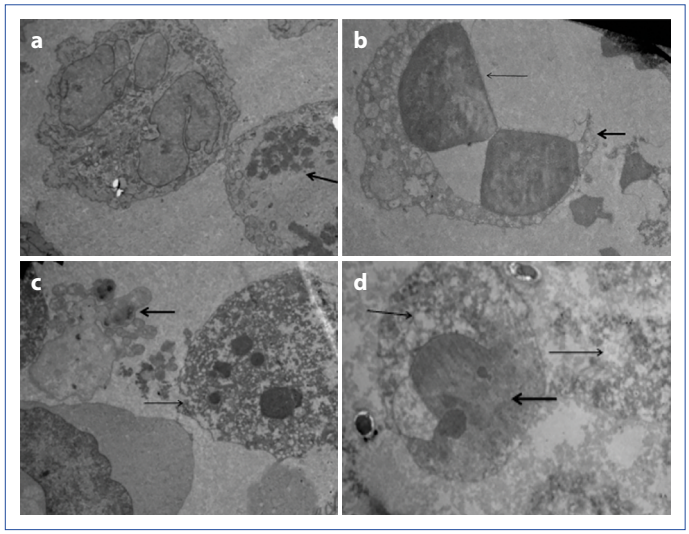

Figure 4. Transmission electron microscopy images of acute promyelocytic leukemia (HL-60) cells after 72 hours. (a) HL-60 cell culture control group image displaying healthy mitotic metaphase cells (arrow) with continuity of both multilobed nucleus and cell membranes (Zoom ×5000). (b) Sorafenib group cell culture shows disrupted cell membrane, no cell integrity (bold arrow), and multilobed nucleus (thin arrow) (Zoom ×5000). (c) Lithium chloride group cells reveal disrupted cell membrane, no cell integrity (thin arrow), and cell cytoplasm showing apoptotic morphologies (bold arrow). Advanced lytic cytoplasmic damage is visible (Zoom ×5000). (d) Advanced lytic cytoplasmic damage (thin arrow) and nuclear envelope damage (bold arrow) were evident in the combination sorafenib and lithium chloride group (Zoom ×6000).

TEM evaluation of the effects on cell ultrastructure

An electron microscopic image of the HL-60 cell culture control group revealed healthy mitotic metaphase cells with continuity of a multilobed nucleus and cell membranes (Fig. 4a). TEM images of the SOR-only group disclosed a disrupted cell membrane, no cell integrity, and a multilobed nucleus. Advanced lytic cytoplasmic damage was visible. (Fig. 4b). LiCl group images showed a disrupted cell membrane, no cell integrity, and cell cytoplasm with apoptotic morphology. Advanced-level lytic cytoplasmic damage in cells was observed (Fig. 4c). In the combination LiCl+SOR group HL-60 cell culture, advanced level lytic cytoplasmic damage and nuclear envelope damage were evident (Fig. 4d).

Discussion

This study explored the combined use of SOR and LiCl to examine the possibility of synergistic effects when administered together. Our results demonstrated that SOR and LiCl inhibited cell proliferation and induced apoptosis in HL-60 cells. Both drugs seemed to demonstrate these effects via the caspase-3 pathway, GSK-3β, and decreased p-AKT and p-p38 levels. We found a time-dependent decrease in tumor cell proliferation with the administration of the drugs, and

binary drug therapy caused the greatest reduction. SOR applied to APL cells has been reported to reduce the number of cells [3, 16-19]. Consistent with our findings, lithium preventing carcinogenesis in APL cells and various cell lines has also been seen in current studies in the literature [20, 21, 15]. The effect of increasing apoptosis was stronger than the effect of decreasing total cell count. LiCl was found to have a greater effect on decreasing the number of tumor cells and increasing apoptosis. Our study was also consistent with previous results reporting the detection of annexin V-positive cells using flow cytometry, which found evidence of apoptosis induced by SOR in APL cell lines [3, 16-18], as well as other cell lines [22-23]. Our results also comported with other reports of the expression of annexin V-positive cells that showed apoptosis induced by LiCl in APL cells [20-21], and other cell lines [15].

There are 4 mechanisms of cell death: apoptosis, necrosis, autophagy, and mitotic catastrophe. An increased caspase-3 level differentiates apoptosis from other types of cell death [24-26]. There is a direct correlation between the activation of caspase-3 and the formation of apoptosis [24]. Both SOR and LiCl stimulate apoptosis through caspase-3 activity. In this study, when the drugs were compared individually, LiCl increased caspase-3 activity more than SOR, which was consistent with greater apoptosis findings compared with SOR. In a study published by Ki et al. [27], it was confirmed that apoptosis occurred via the caspase-linked pathway and that drug-induced apoptosis was reduced when a caspase-3 inhibitor was added to HL-60 cells. Our study with leukemic cell lines treated with SOR revealed increased caspase-3 activation, which was consistent with previous results [3, 17, 19]. LiCl applied to leukemic cell lines has also been demonstrated to prompt an apoptotic response through increased caspase-3 activation [21].

TEM results revealed that while the cell membrane and nucleus structures in the control group were preserved, cell integrity was impaired in the drug-treated groups. This was more pronounced in the LiCl group than in the SOR group. The increase in apoptosis detected with flow cytometry was confirmed microscopically. In the LiCl group, the integrity of the cell membrane was not maintained, the cytoplasm of some cells reflected apoptotic morphology, and the majority of cells reflected advanced lytic cytoplasmic changes, which was also reflected in histograms demonstrating apoptosis. In the SOR group, it was observed that the cell membrane was damaged and did not maintain its integrity, the core material was separated into lobes, and there were advanced lytic changes in the cytoplasm. In the combination group, severe lytic cytoplasmic damage and nuclear membrane damage were observed in the cells. These results also support the cell count and apoptosis results.

The inhibitory effect of SOR on p-GSK-3β has previously been reported in the literature [28]. In our study, ELISA method measurements indicated that SOR caused a more significant decrease in p-GSK-3β values than LiCl. In a study performed by Panka et al. [29], it was found that the use of GSK-3β inhibitors increased the effect of SOR in melanoma cell cultures. Wang et al. [16, 18] demonstrated that SOR inhibited GSK-3β phosphorylation in HL-60 cells and decreased the level of the antiapoptotic myeloid cell leukemia 1 (Mcl-1) protein, consequently increasing the inhibitory effect of apoptosis-inducing drugs.

When GSK-3β is phosphorylated as a structure, it changes to an inactive form. Increased levels of p-GSK-3β in the LiCl group compared with the control group in this study also confirmed that LiCl influenced this pathway. LiCl was shown to promote apoptosis via the AKT signaling pathway in a study where LiCl was added to APL cells, resulting in a decrease in the p-GSK-3B level and the AKT protein level [20]. In another study, the anti-leukemic effect of LiCl on APL cells was demonstrated in an increase of p- ERK1/2 induced by GSK-3β inhibition [21].

AKT is responsible for cell survival. Other studies have found that p-AKT, the active form of AKT, was reduced during apoptosis induced by SOR, [19, 30]. Edwards et al. [31] investigated the potential inhibitory activity of SOR, including inactivation of GSK-3β. They found that SOR partially reduced p-AKT, which increased GSK-3β inactivation. The addition of an AKT inhibitor led to a decrease in p-GSK-3β and confirmed that GSK-3β was a kinase of the PI3K/AKT pathway. It is useful to try to add parameters that will illustrate this relationship. Hu et al. [32] found that SOR induced apoptosis and that p-AKT levels did not change in a study of an AML cell line. Wang et al. [18] found that the addition of AKT inhibitors decreased the level of Mcl-1 in HL-60 cells, leading to an increase in ATO-induced apoptosis.

The greatest decrease in this study of the p38 molecule in the MAPK pathway was seen in the p-p38 level in the SOR-only group. SOR was also found to reduce the level of p38 in another study that examined SOR p38 MAPK activation [31]. LiCl has also been reported to reduce p-38 MAPK phosphorylation [33-35].

In our study, it was found that SOR decreased p-ERK levels compared with the control group, but the result was not statistically significant. Moreover, LiCl and dual drug therapy did not significantly change p-ERK levels. In studies evaluating the ERK level during apoptosis induced by SOR, it was found that p-ERK, the active form, decreased [3,16]. Edwards et al. [31] reported that SOR did not have a significant effect on ERK activity. In a study evaluating LiCl treatment, an increased p-ERK1/2 level had an antitumoral effect [21].

In our study, it was found that SOR, LiCl, and the combination of these 2 drugs did not significantly change p-IκBα levels. NF-κB is the inactive form of IκB and IκBα has a high binding affinity for the p65 subunit. This results in activation of IκB kinase (IKK) as a result of physiological and pathological stimuli to the cell [36]. Activated IKK phosphorylates the IκB protein. After ubiquitination due to phosphorylation, IκBα undergoes

proteolysis. NF-κB then advances to the nucleus and stimulates target genes. Genes under NF-κB control are associated with tumor progression, apoptosis, and metastasis [37]. Wu et al. [23] found an increase in NF-κBp65 and IKKβ, and a decrease in IκBα, components of the NF-κB pathway of cancer cells. Thus, it has been demonstrated that NF-κB is activated in cancer cells and inhibition of NF-κB increases the effect of SOR on cell death. This study is one of the few in the literature to demonstrate the relationship between SOR, apoptosis, and IκBα [23]. Another study found that SOR had an inhibitory effect on NF-κB, a transcription factor [38]. There is only a study published in 2002 that demonstrates the relationship between LiCl, apoptosis and IκBα. According to that study, GSK-3β plays a role in the transactivation of NF-κB by phosphorylating the p65 subunit from the C-terminal, NF-κB protects cells against apoptosis, and when GSK-3β was inhibited pharmacologically (increase in IκBα degradation) with LiCl, IκBα levels were decreased, but the result was not statistically significant compared with controls. The effect of GSK-3β was thought to be independent of NF-κB [39]. Other authors found that inhibition of GSK-3β by LiCl reduced NF-κB activation, which accelerated caspase 8-mediated apoptosis [40]. In a study on colorectal cancer cells, it was reported that LiCl treatment reduced NF-κB levels and stimulated apoptosis in cells by affecting the B-cell lymphoma gene and other surviving genes [15].

When the effects of SOR and LiCl on p-c-Jun were evaluated individually and compared with the control group, we found that LiCl caused a statistically significant decrease. Different studies using LiCl for therapeutic purposes have shown that it reduced p-c-Jun levels and phosphorylation [33, 41]. It has been reported that p-c-Jun levels decreased in SOR-treated cells and were high in SOR-resistant cells [43]. Lin et al. [44] reported that apoptosis induced by SOR and melatonin was reduced when a JNK/c-Jun pathway inhibitor was added [44].

The levels of p-STAT3 in our study were below a level that was detectable, and therefore, no evaluation could be performed in any of the groups. However, previous studies have shown that SOR reduces the level of p-STAT3 [42, 30].

In this study, there was a decrease in p-p38 and p-AKT levels in all of the signal transduction parameters, which may indicate that this pathway is important with respect to the effects of SOR and LiCl. SOR, which is known to act via the Ras/Raf/MEK/ERK signal transduction pathway, has been shown to reduce p-ERK levels in the literature [3, 16, 18]. Our study results were not consistent. We observed that apoptosis increased significantly when SOR and LiCl were administered together, and we concluded that the drugs have a synergistic effect. The effects of LiCl, which inhibits protein kinase C and GSK-3β, and SOR, which inhibits the Ras/Raf/MEK/ERK cascade, are interrelated mechanisms. We think it would be useful to repeat the study with additional inhibitors of these proteins to clarify the interrelated mechanisms.

SOR and LiCl may be considered complementary treatment options for standard APL treatment. In addition, the psychiatric disorders often seen in patients diagnosed with APL may further support the use of LiCl in these patients.

Conclusion

The results of this study indicated that SOR and LiCl significantly reduced the number of tumor cells and increased the apoptosis rate in in vitro HL-60 human APL cell cultures. Nonetheless, further research is necessary before this can be applied to routine APL treatment.

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Conflict of interest: There is no conflict of interest between the authors.

Ethics Committee Approval: This study was conducted in cell culture, so ethics committee approval is not required.

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Authorship contributions: Concept – A.E.; Design – A.E.; Supervision – S.K.; Data collection &/or processing – A.E.; Analysis and/or interpretation – A.E., C.E.; Literature search – A.E.; Writing – A.E.; Critical review – S.K.

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