Medical Oncology (2025) 42:326
https://doi.org/10.1007/s12032-025-02898-9

ORIGINAL PAPER

Effects of 3-Bromo-2-oxopropionic acid and LiCl treatment combined with electroporation on apoptotic and metabolic responses in DLD-1 colon cancer cells

Güney Gürsoy[1] · **Zehra Çiçek**[2]

Received: 11 March 2025 / Accepted: 25 June 2025 / Published online: 12 July 2025

Abstract

Colon cancer is particularly increasing in incidence in developed countries, and it ranks first in terms of morbidity and mortality worldwide. As in other types of cancer, metabolic changes and cellular death mechanisms play a critical role in cancer treatment. Cancer cells prefer glycolysis for energy production even in the presence of oxygen; this phenomenon is known as the Warburg effect. Glycolysis, which is essentially an anaerobic process, provides metabolic adaptation by preventing mitochondrial oxidative phosphorylation in cancer cells. Electroporation (EP) is a method that increases the uptake of drugs into the cell by creating temporary pores in the cell membrane. 3-Bromo-2-oxopropionic acid and Lithium chloride (LiCl) are agents that may show anticancer potential through different mechanisms. The aim of this study was to investigate the potential anticancer effects of the EP-assisted combination of 3-Bromo-2-oxopropionic acid and LiCl agents in DLD-1 colon cancer cells and to evaluate how these effects occur at the level of cell viability, apoptotic activity and metabolic markers. In the study, cell viability was evaluated by WST-8 tetrazolium based colorimetric method. Within the scope of analysis of apoptotic activity and metabolic responses; caspase-3, Phosphatidylinositol 3-kinase (PI3K) and Glucose transporter-1 (GLUT-1) levels were determined by ELISA method. While no change in cell viability was observed at 10 and 20 µM doses of 3-Bromo-2-oxopropionic acid, a significant decrease was detected in its combination with EP. IC_{50} value was determined as 37.99 ± 2.34 µM in a single application and as 27.33 ± 1.99 µM in its combination with EP. While low doses of LiCl (5 and 10 mM) did not affect cell viability, a significant decrease was detected at doses of 50, 75 and 100 mM. In its combination with EP, a significant loss of viability was observed even at low doses and the IC_{50} value decreased from 98.92 ± 0.64 mM to 81.88 ± 1.64 mM. A decrease in IC_{50} doses is observed in the treatment with the combination of both 3-Bromo-2-oxopropionic acid and LiCl with EP. The combined treatment enables the same lethal effect on cancer cells with lower drug doses. Caspase-3 activity increased significantly in combined applications with EP. While 3-Bromo-2-oxopropionic acid alone did not change the level of PI3K, it caused a significant increase in its combination with EP. While LiCl caused an increase in the level of PI3K at low doses, a decrease was detected in its combination with EP. Regular partial increases in GLUT-1 level were determined in a dose-dependent manner in 3-Bromo-2-oxopropionic acid treatment alone. A similar increase was determined at 20 and 30 µM doses combined with EP, while a decrease occurred at 40 µM. While no significant increase was detected in GLUT-1 levels compared to the control group in 10 mM LiCl treatment alone and in combination with EP, a significant increase was detected in 50 and 75 mM LiCl doses compared to the control group. In conclusion, 3-Bromo-2-oxopropionic acid and LiCl show anticancer potential by affecting apoptotic and metabolic processes, and their combination with EP increases these effects. In order to explain the effectiveness of these treatment approaches from every perspective, it is recommended to conduct in vitro studies on different colon cancer cell lines and evaluate them with more comprehensive in vivo and clinical studies.

Keywords Electroporation · 3-Bromo-2-oxopropionic acid · Lithium chloride · Caspase-3 activity · Metabolic markers · Anticancer treatment strategies

Extended author information available on the last page of the article

Introduction

Colon cancer is the most common type of gastrointestinal cancer and is the third most common type of cancer worldwide. However, it is among the top three leading causes of cancer-related deaths [1]. Colon cancer is a disease that is caused by interactions between environmental factors and genetic effects [2]. In a large majority of colon cancer cases, it develops from colorectal polyps, which is one of the places where gastrointestinal polyps are frequently seen, and the formation of adenomatous polyps usually leads to colon cancer [3, 4]. Cancer cells require high glucose uptake and glycolysis activity to sustain their survival. This metabolic adaptation, known as the Warburg effect, enables rapid energy production by keeping the glycolysis pathway active even in the presence of oxygen [5–8]. Hexokinase (HK) is an enzyme that phosphorylates glucose to glucose-6-phosphate (G6P) and initiates the process of glycolysis, and is found at high levels in cancer cells. As a central intermediate in metabolic pathways required for ATP production and anabolic processes, G6P plays a critical role in meeting the rapid energy and biosynthesis needs of tumor cells. This supports the growth and survival of cancer cells [7–9]. Hexokinase II (HKII) is the most active isoenzyme of the HK family and its overexpression is associated with poor prognosis and resistance to treatment in different malignancies. In cancer cells, HKII acts as a kind of anti-apoptotic regulator in the cell, directly related to ATP production. Similarly, studies on colon cancer have reported that the increase in HKII is associated with negative effects and tumor progression [9–13]. Therefore, HK inhibitors suppress glycolysis, reduce ATP production and thus trigger apoptosis by limiting energy resources in cancer cells and are considered a potential target for cancer therapy [7, 9, 14–16]. 3-Bromapyruvate (3-BP), a HK inhibitor, is a promising anticancer agent that shows significant efficacy in many types of cancer by targeting many important points in cancer biomechanism [17–20]. 3-BP inhibits glycolysis in cancer cells, which reduces ATP production and causes cells to deplete their energy. It targets hexokinase II, which increases ROS, which disrupts mitochondria and initiates the intrinsic apoptosis process. It can also cause necroptosis in some cells and alter the autophagy pathway. Due to these multiple effects, 3-BP is thought to be an effective anticancer agent for glycolysis-dependent and treatment-resistant cancers [20–24].

Lithium has been widely used for years in the treatment of mental health disorders such as bipolar disorder and psychosis. The effect of lithium is mainly expressed by two mechanisms: inositol depletion and glycogen synthase kinase-3 inhibition hypothesis [25, 26]. Overexpression of glycogen synthase kinase-3 in particular is observed in different types of cancer. This has led lithium to be considered as a potential anticancer agent in cancer treatment [27, 28]. Lithium, while primarily recognized as a GSK-3β inhibitor, demonstrates diverse anticancer effects in cancer cells. This process regulates Wnt/β-catenin signaling through the inhibition of GSK-3β, modulates p53 activity, and promotes apoptosis [29–31]. Lithium induces apoptosis through the mitochondrial pathway by modifying the Bax/Bcl-2 balance and enhances extrinsic apoptosis by elevating TRAIL receptors. In addition, it arrests the cell cycle in the G2/M phase, stimulates autophagy, and promotes cell differentiation in some hematological cancers [29, 32–35]. These multiple effects make lithium an agent with high therapeutic potential.

Surgery, chemotherapy and radiotherapy are among the basic clinical treatments for colon cancer, but there are also different treatment approaches such as immunotherapy. However, there are different restrictions for each treatment method [36–40]. Electroporation is a method in which membrane permeability is increased by temporarily changing the membrane integrity of DNA, RNA, GEN and drugs that cannot pass through the cell membrane. Pulsed and high-amplitude electric field applied to cells and tissues allows the formation of aqueous pore structures in the membrane. The effectiveness of the application is closely related to the electroporation parameters and varies depending on the molecule to be transferred [41–43]. Electroporation application can be adjusted with time, frequency, waveform and pulse amplitude parameters. The fraction of area electroporated is related to the pulse amplitude, while the duration and number of pulses are related to the pore density. Depending on the change in the structure of the cell membrane, electroporation application creates a reversible or irreversible situation [44–47]. Reversible electroporation is a temporary high conductivity state of the membrane and enables a wide range of applications. It allows the transfer of drugs, genes and other molecules into the cell under conditions where the cells maintain their viability. The combined use of reversible electroporation with drugs used in chemotherapy is called electrochemotherapy. With the application of electrochemotherapy, maximum cytotoxic effect is achieved at low drug doses [48–51]. Many chemotherapeutic drugs such as bleomycin and cisplatin, which are commonly used in chemotherapy, have side effects that negatively affect the quality of life. With electrochemotherapy, the effects are achieved using lower doses of medication [52–55].

The aim of this study was to evaluate the potential anticancer effects of 3-Bromo-2-oxopropionic acid and lithium chloride (LiCl) agents on DLD-1 colorectal cancer cells when used in combination with EP. Electroporation parameters were selected in accordance with the ESOPE protocol to temporarily increase the permeability of the cell membrane

and were used in EP applications. In the study, the effects of single and combined treatment groups on cell viability, as well as cell death mechanisms and metabolic responses were discussed together. Apoptotic response was evaluated via intracellular Caspase-3 activity; whether the observed cell death was limited to apoptosis was also discussed within the framework of the results. Phosphatidylinositol 3-kinase (PI3K) and Glucose Transporter-1 (GLUT-1) levels, which play an important role in the metabolic activity of cancer cells, were quantitatively analyzed at the protein level by ELISA. The selection of these markers was based on their relationship with basic biological processes such as energy metabolism, survival and resistance to treatment in tumor cells. In this context, the study evaluates the cytotoxic, apoptotic and metabolic effects of 3-BP and LiCl when applied alone and together with EP, and aims to provide preliminary data on the anticancer potential of these agents by shedding light on their possible molecular mechanisms of action.

Materials and methods

Preparation of cell

The DLD-1 cell line used in the study was obtained from the University of Health Sciences Gülhane Stem Cell Laboratory. Cells were cultured in 75 cm^2 culture flasks in RPMI 1640 medium (Capricorn scientific, Cat no: RPMI-A, Germany) with 10% fetal bovine serum (FBS, Capricorn scientific, Cat no: FBS-11A, Germany) and 1% penicillin/streptomycin antibiotic (PSA, Gibco, Thermo Fisher Scientific, Cat no: 15140122, USA). Experiments were started when the cells kept in a cell culture environment (37 °C in an incubator with 5% CO_2, PHcbi, Japan) in a 75 cm^2 flask reached sufficient density. 5 mL of Dulbecco's Phosphate buffered saline (DPBS, Capricorn scientific, Cat no: PBS-1A, Germany) was added into the flask, washing was performed and aspirated. To remove the adherent cells at the bottom of the flask, 4 mL trypsin/EDTA (Biowest, Cat no: L0930 France) was added to the 75 cm^2 flasks and the cells were incubated for 1–2 min. It was checked with an inverted microscope whether the cells were lifted off the surface. To inactivate trypsin, cell medium was added twice the amount of trypsin. The cell suspension in the flask was transferred to the falcon tube and centrifuged at 1200 rpm for 3 min and the supernatant was removed. Cells were made ready for use by adding 5 mL of cell medium to the falcon tube.

Application of electroporation

The suspension at a density of 1×10^5 cells/100µL was placed in EP cuvettes with 4 mm spacing. Cuvettes were placed in the EP chamber in a BTX square wave pulse generator (BTX ECM 830). The control group was placed in the EP tub and placed in the EP chamber, but no electrical pulse was applied. EP parameters; EP application was performed with a repetition frequency of 1 Hz, 8 square wave pulses, 100 µs and electric field amplitudes between 0 and 2000 V/cm. The experiments were repeated three times. Following EP treatment, 1×10^4 cells were seeded in 96-well plates and incubated briefly (approximately 10 min) in appropriate culture medium for cell viability analysis (Fig. 1).

Chemical treatment of DLD-1 cells with 3-Bromo-2-oxopropionic acid and LiCl

After the cell preparation step, the cells in the falcon tube were seeded into 96-well plates with 1×10^4 cells per well and incubated for 24 h. After incubation, DLD-1 cells were treated with the determined concentrations of 3-Bromo-2-oxopropionic acid (0 (Control), 10, 20, 30, 40 and 60 µM), (CAS number: 1113-59-3, Sigma-Aldrich, USA) and LiCl (0 (Control) 5, 10, 75 and 100 mM), (CAS number: 7447-41-8, Merck, Germany) and incubated for 24 h in an incubator containing 5% CO_2 at 37 °C (Fig. 1).

Cell viability assay

Electroporation alone, with 3-Bromo-2-oxopropionic acid and LiCl, and with EP combined chemicals were applied and cells were seeded into plates as 100 µL. After incubating the cells in culture medium for one day, 10 µL of cell proliferation reagent WST-8 (Cat number: A014-1, ABP Biosciences, USA) was added to each well to determine cell viability and after approximately three hours, absorbance values were measured with an ELISA reader spectrophotometer at the appropriate wavelength (450 nm) (Molecular Devices Filter Max F5, Japan). The results are presented as percentage of cell viability compared to the control group (Fig. 1).

Caspase-3 activity analysis

After synergistic doses of 3-Bromo-2-oxopropionic acid, LiCl and electroporation (EP) combinations were applied, caspase-3 activity was evaluated from the obtained cell suspensions by fluorometric method. 1×10^6 cells from each group were seeded in six-well plates and incubated for 24 h. Then, the medium was removed, 1000 µL of 0.25% trypsin/EDTA was added to each well and incubated for 90 s. After the separation of the cells was observed with a microscope, 2000 µL of complete cell culture medium was added and the suspension was transferred to Eppendorf tubes. The samples were centrifuged at 300 g for 3 min to remove the supernatant, the pellets were washed with 500 µL of PBS and centrifuged again in the same way. Cell pellets were

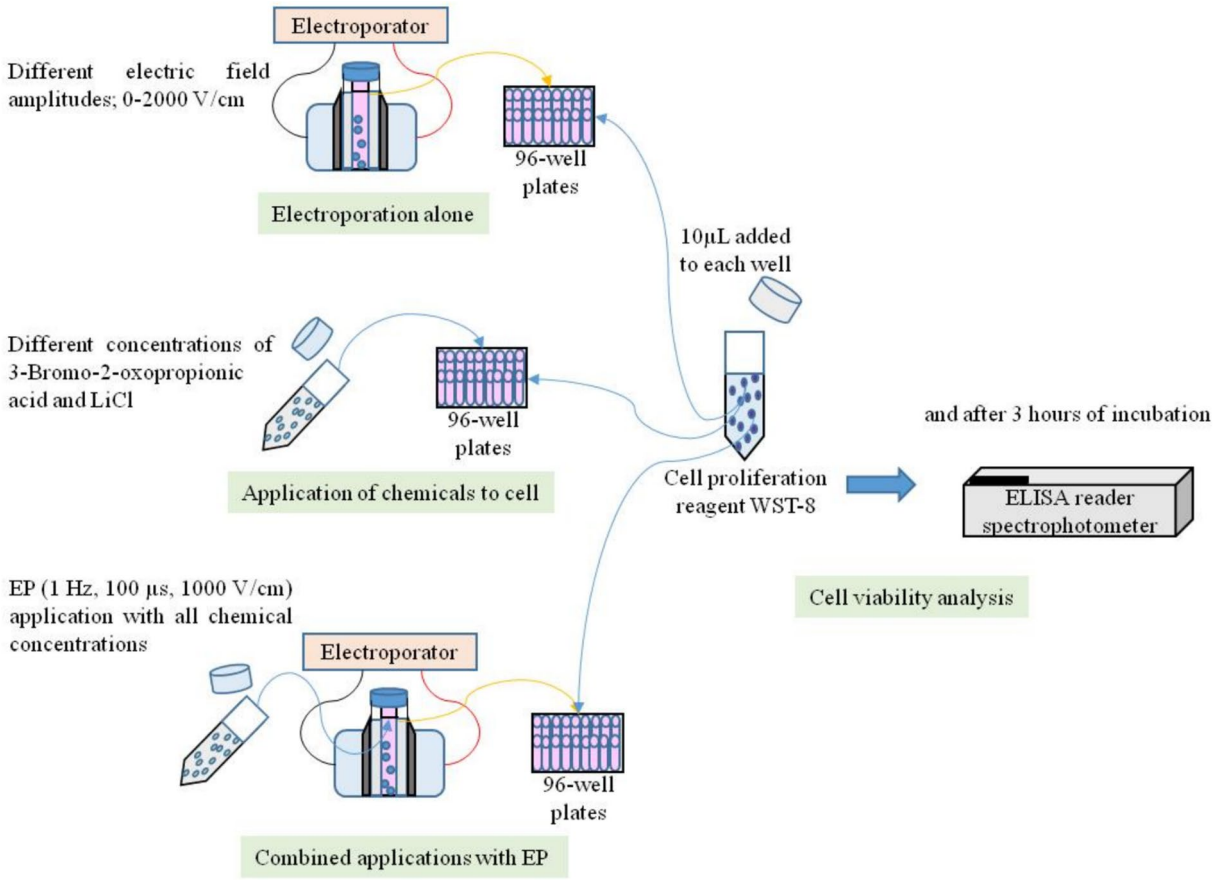

Fig. 1 Schematic representation of 3-Bromo-2-oxopropionic acid and LiCl applications alone and combined with electroporation

incubated with 50 µL of cell lysis buffer at 4 °C, incubated in the refrigerator for 10 min, and then centrifuged at 10,000 g for 10 min. Total protein amounts of the lysates were measured by the BCA method and compared with bovine serum albumin (BSA) standard. Caspase-3 activity was determined using a fluorometric assay kit (Caspase-3/CPP32 Fluorometric Assay Kit, Cat number:#K105-25, BioVision, USA) according to the manufacturer's protocol. Measurements were made on the basis of fluorescence intensity (Ex/Em = 400/505 nm) with a plate reader Molecular Devices Filter Max F5. Results were expressed as percentage of fluorescence intensity relative to the control.

Analysis of PI3K protein level

Phosphatidylinositol 3-kinase (PI3K) is an important signaling pathway component that plays a role in cellular proliferation, metabolic activity and survival. In this study, PI3K levels were determined by ELISA method in cell lysates obtained after synergistic combinations of 3-Bromo-2-oxopropionic acid, LiCl and EP. Cell collection, lysis and total protein determination procedures were performed by BCA method. Measurements were performed using Human PI3K ELISA Kit from ELK Biotechnology company (Human PI3K (Phosphotylinosital 3 kinase), ELISA Kit, Cat number: ELK9879, ELK Biotechnology, USA), in accordance with the manufacturer's protocol. Absorbance values were measured at 450 nm, and the results were presented in ng/mg total protein unit.

Analysis of GLUT-1 protein level

GLUT-1 is a glucose transporter protein that is frequently overexpressed in tumor cells to meet the increased glucose demand. In this study, GLUT-1 levels were measured using the same cell lysates using the Human GLUT-1 ELISA Kit from Elabscience (Human GLUT1(Glucose Transporter 1) ELISA Kit, Cat number:E-EL-H1822, Elabscience, USA). Measurements were performed spectrophotometrically at 450 nm and data were analyzed according to the standard curve. GLUT-1 levels were normalized to the total protein amount and reported as ng/mg protein. This analysis was performed to evaluate the effects of applied treatments on the metabolic reprogramming of cells.

Statistical analysis of data

Statistical analyses were performed using GraphPad Prism 9 software. All experiments were repeated three times and the data obtained were reported as mean ± standard deviation (mean ± SD). Comparison of data with the control group was performed using one-way ANOVA test; Tukey's test was used as post-hoc in this analysis. Two-way ANOVA analysis was performed to evaluate differences between multiple groups. Statistical significance was determined as $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$.

Results

Cellular responses dependent on electroporation field amplitude: viability analysis in DLD-1 cells

Viability changes in DLD-1 cells were determined after short-term incubation using 100 µs, 1 Hz and 8 square wave electroporation parameters together with varying electric field intensities. No significant change was observed in viability compared to the control group up to 400 V/cm electric field amplitude ($p > 0.05$). At 800 V/cm amplitude, partial proliferation occurred in the cells ($p < 0.05$). A partial decrease in cell viability was observed at 1000 V/cm electric field amplitude determined in the protocol standardized by the European Electrochemotherapy Standard Operating Procedure (ESOPE) for reversible electroporation, but this was not statistically significant ($p > 0.05$) [56, 57]. At 1200 V/cm amplitude, cell viability was found to be 87.09% and this electric field value was determined as a critical point for the decrease in viability ($p < 0.05$). With the increase in electric field amplitude, cell viability was found to be 59.02% at 1600 V/cm and 47.03% at the maximum field amplitude of 2000 V/cm ($p < 0.001$) (Fig. 2).

The effects of 3-Bromo-2-oxopropionic acid alone, LiCl and EP and their combinations on DLD-1 cell viability are presented in Fig. 3. EP parameter was selected as 100 µs, 1 Hz 8 square waves and 1000 V/cm according to ESOPE standards. EP application alone (EP control) was found to cause a partial decrease in cell viability, but this decrease was not statistically significant compared to the control group ($p > 0.05$).

Effects of 3-Bromo-2-oxopropionic acid in combination with electroporation on cell viability

In the application of 10 and 20 µM 3-Bromo-2-oxopropionic acid alone, no statistically significant change was observed in cell viability compared to the control group ($p > 0.05$). On the other hand, in the application of 20 µM 3-Bromo-2-oxopropionic acid combined with EP, cell viability was determined as 65.38% and this dramatic decrease was seen to be statistically significant compared to both control groups ($p < 0.05$, $p < 0.001$). In the applications of 30, 40 and 60 µM -Bromo-2-oxopropionic acid, cell viabilities were determined as 72.1%, 43.98% and 11.56%, respectively. Cell viability tended to decrease in a correlation with increasing doses and the decrease in viability compared to the control group was statistically significant ($p < 0.001$) (Fig. 3a). The concentration value that inhibited half of DLD-1 cells for 3-Bromo-2-oxopropionic acid was found as $IC_{50} = 37.99 \pm 2.34$ µM. In combined applications with EP, the decrease in viability was more pronounced at concentrations of 20, 30 and

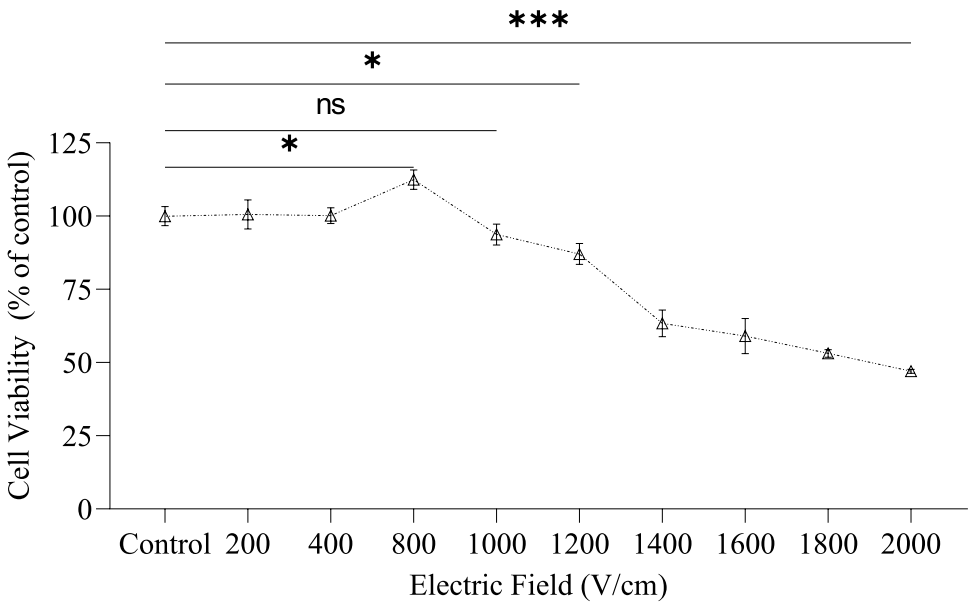

Fig. 2 Effect of electroporation on cell viability in DLD-1 cells: The role of electric field amplitude (ns: not significant, $*p < 0.05$ and $***p < 0.001$)

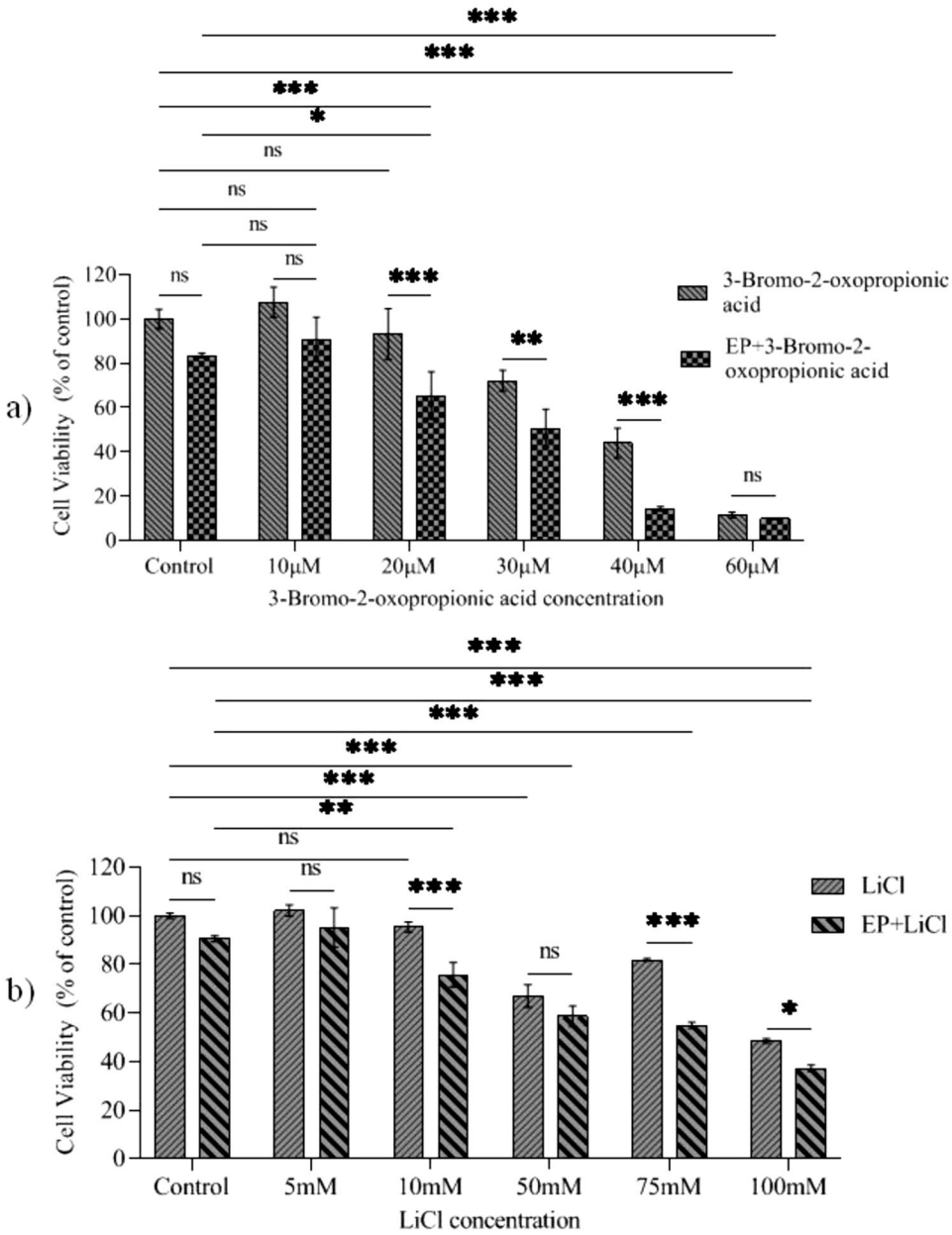

Fig. 3 Effects of 3-Bromo-2-oxopropionic Acid and LiCl applications alone and in combination with electroporation on cell viability (ns: not significant, $*p<0.05$, $**p<0.01$ and $***p<0.001$)

40 μM and when compared with the groups when these doses were applied alone, it was seen that the effectiveness of EP in decreasing viability showed a significant change ($p<0.001$, $p<0.01$, $p<0.001$). In the combined treatment of 3-Bromo-2-oxopropionic acid with EP, $IC_{50}=27.33\pm1.99$ μM (Fig. 3a). The morphology of cells was visualized with an inverted light microscope after single dose applications of 3-Bromo-2-oxopropionic acid and combined treatments with EP. It was observed that at the same dose values, combined treatment with EP caused changes in the structural form of the cells along with the decrease in cell viability (e.g., 30 μM alone and EP + 30 μM) (Fig. 4a).

Effects of LiCl and electroporation combination on cell viability

No significant change was observed in the viability of DLD-1 cells in 5 and 10 mM LiCl alone application compared to the control group ($p>0.05$). A statistically significant decrease was found in cell viability in increasing doses of LiCl compared to the control group ($p<0.001$) (Fig. 3b). $IC_{50}=98.92\pm0.64$ mM was determined in LiCl alone application. In combination with EP, cell viability decreased to 75.71% at a dose of 10 mM LiCl and this change was statistically significant compared to both control groups ($p<0.001$, $p<0.05$). For increasing LiCl doses, the decrease in cell

Fig. 4 Inverted microscope (Nikon Eclipse TS100) images of the effects of 3-Bromo-2-oxopropionic acid and LiCl alone and together with electroporation on cell morphology at × 200 (**a**) and × 100 (**b**) magnifications

viability became more pronounced in combined applications with EP ($p < 0.001$). In combined treatments of 75 and 100 mM LiCl with EP, cell viabilities were found to be 54.86% and 37.22%, respectively. When applications of 10, 75 and 100 mM LiCl alone and EP combinations were compared, it was determined that the combined application had a statistically significant effect ($p < 0.001$, $p < 0.001$, $p < 0.05$). In the combined treatment of LiCl with EP, $IC_{50} = 81.88 \pm 1.64$ mM was found (Fig. 3b). In Fig. 4b, the appearance of DLD-1 cells after combined treatment with LiCl and EP alone is presented with an inverted light microscope. It was determined that deformation occurred in the cell structure at increasing LiCl doses, and changes in the morphological structure at the same dose values were observed in the combined treatment with EP.

Effects of combined application of 3-Bromo-2-oxopropionic acid and LiCl with electroporation on caspase-3 activity

The changes in caspase-3 activity in DLD-1 cells with the combinations of 3-Bromo-2-oxopropionic acid, LiCl and EP are given in Fig. 5a, b. There was no significant change in caspase-3 activity at 20, 30 and 40 µM

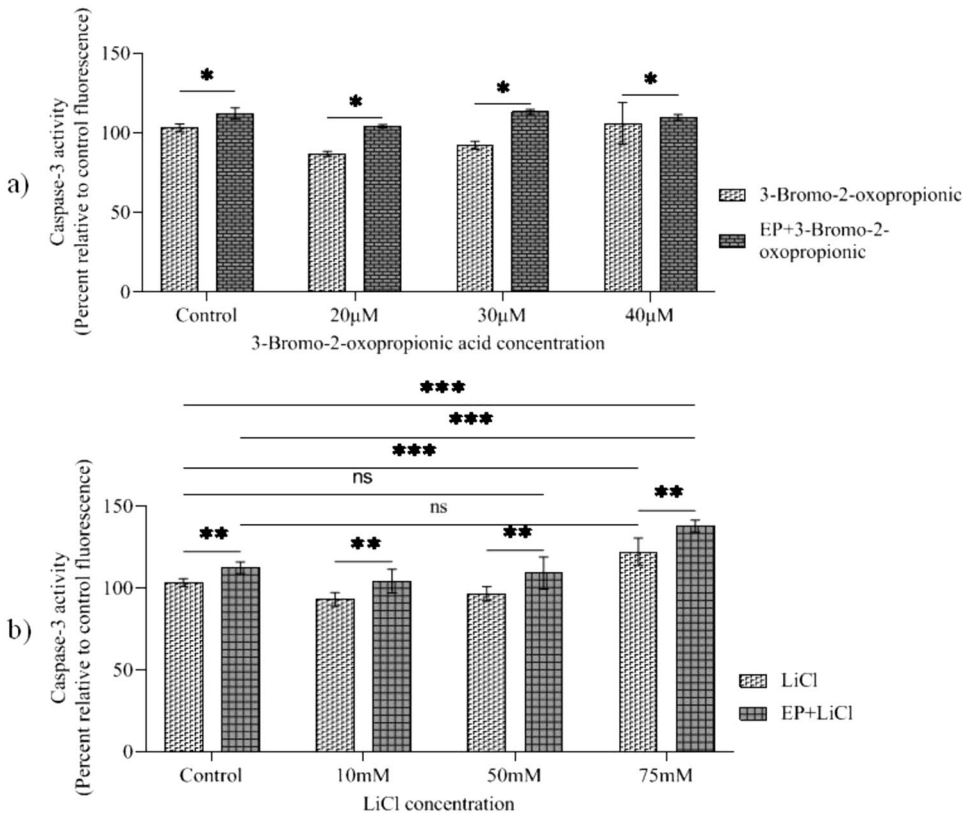

Fig. 5 Effects of 3-Bromo-2-oxopropionic acid and LiCl treatments alone and in combination with electroporation on caspase-3 activity as an indicator of apoptosis in DLD-1 cells (ns: not significant, $*p<0.05$, $**p<0.01$ and $***p<0.001$)

3-Bromo-2-oxopropionic acid concentrations compared to the control group ($p>0.05$). It was found that EP application alone caused a partial increase in caspase-3 activity and this increase was determined to be a statistically significant change compared to the control group ($p<0.05$). In addition, it was determined that caspase-3 activity increased in the combined treatment with EP for all concentration values, but it was not found to be statistically significant when compared to the control group ($p>005$) (Fig. 5a). On the other hand, when caspase-3 activities of single and combined applications were compared, it was seen that EP combined treatments increased caspase-3 activity ($p<005$). It was found that there were partial increases in caspase-3 activity in the groups treated with 10 and 50 mM concentrations of LiCl alone and in combination with EP, but these changes were not statistically significant ($p>0.05$). On the other hand, it was found that caspase-3 activity increased at a concentration of 75 mM ($p>0.001$). For the same dose value, caspase-3 activity was found to increase significantly when applied together with EP ($p>0.001$) (Fig. 5b). In addition, when combined applications were compared for all dose values, it was seen that activity increased in the EP groups ($p<0.01$).

Determination of PI3K levels in response to treatment

PI3K levels was determined by ELISA method after combined treatments of DLD-1 cells with 3-Bromo-2-oxopropionic acid, LiCl and EP (Fig. 6). No significant change was observed in PI3K levels compared to the control group at 20 and 30 µM 3-Bromo-2-oxopropionic acid concentrations ($p>0.05$). There was a partial increase compared to the control group at 40 µM concentration ($p<0.05$). A dose-dependent increase in PI3K levels was found in combined treatments with EP at 30 and 40 µM concentrations. It was observed that the combined applications were statistically significant when compared to the single applications of the same doses and the control groups ($p<0.05$, $p<0.01$, $p<0.001$) (Fig. 6a). An increase in PI3K levels was found at 10 and 50 mM LiCl concentrations alone ($p<0.01$). Combined applications of the same dose values with EP showed an increase compared to the control group, but there was no statistically significant change between the single and combined applications ($p>0.05$). In the treatment of 75 mM LiCl alone, there was no statistically significant change in PI3K levels compared to the control group ($p>0.05$). In the

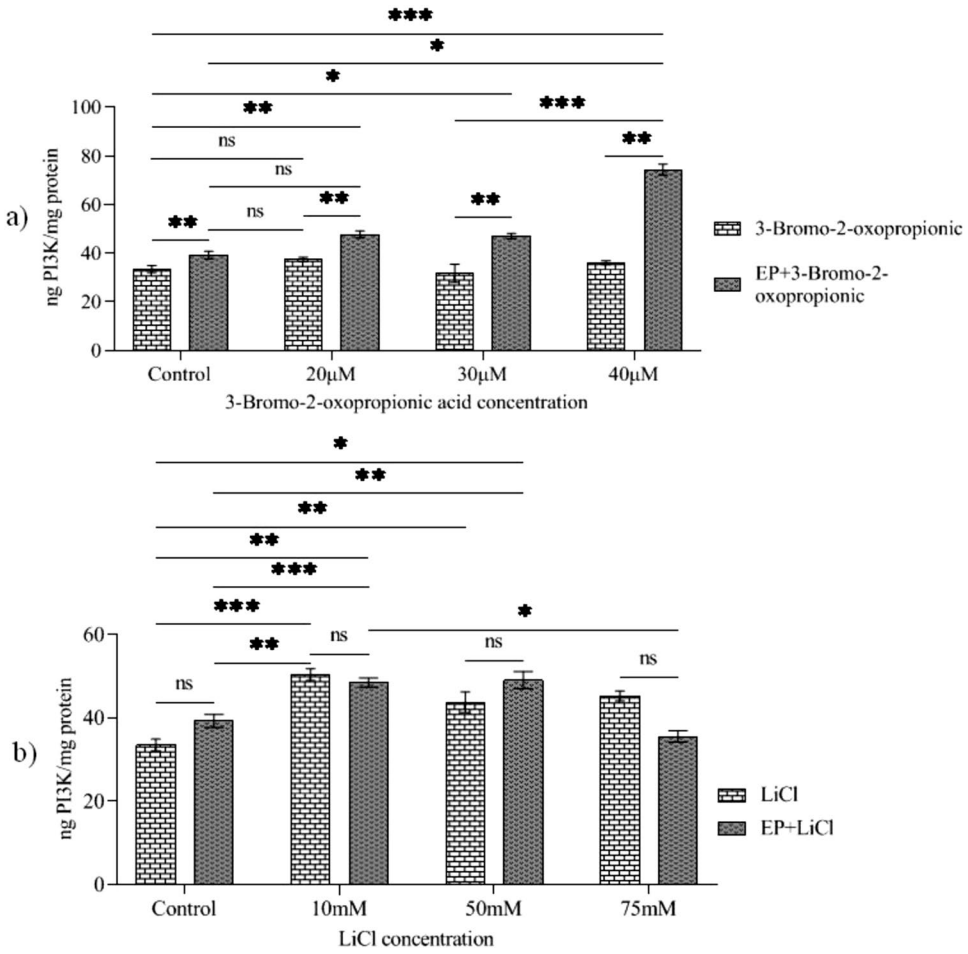

Fig. 6 Effects of 3-Bromo-2-oxopropionic acid and LiCl treatments alone and in combination with electroporation on PI3K levels in DLD-1 cells (ns: not significant, $*p<0.05$, $**p<0.01$ and $***p<0.001$)

combined application with EP, a decrease in PI3K levels was observed compared to the application alone. In addition, when compared to the PI3K levels increase in 10 mM LiCl, the decrease in the high dose was determined to be statistically significant ($p<0.05$) (Fig. 6b).

Determination of GLUT-1 levels in response to treatment

A dose-dependent increase in GLUT-1 levels was observed at all doses of 3-Bromo-2-oxopropionic acid alone. However, this increase was not statistically significant when compared to the control group ($p>0.05$). In combined treatments with EP, an increase in GLUT-1 levels was observed at concentrations of 20 and 30 µM, while a decrease was found at 40 µM. Changes in GLUT-1 levels were not stable in combined treatments with 3-Bromo-2-oxopropionic acid and EP, and no statistically significant difference was observed ($p>0.05$) (Fig. 7a). At the lowest dose selected for LiCl, 10 mM concentration, there was no significant difference in GLUT-1 levels both alone and in combination with EP when compared to the control groups ($p>0.05$). When the concentration was increased to 50 mM, there was a dramatic increase in GLUT-1 levels both alone and in combination with EP. ($p<0.001$). Compared to the control groups, an increase in GLUT-1 levels was observed at 75 mM, while a decrease was observed at 50 mM concentration ($p<0.001$, $p<0.01$) (Fig. 7b).

Discussion

This study demonstrates the apoptotic activity and metabolic responses of 3-Bromo-2-oxopropionic acid and LiCl alone and in combination with EP on human colon cancer cells. 3-Bromo-2-oxopropionic acid, known as a glucose inhibitor, shows its effect on cancer cells by targeting various cell death mechanisms and metabolism. In our study, it was found that 3-Bromo-2-oxopropionic acid caused a dramatic decrease in viability in DLD-1 cells in a dose-dependent manner. The dose-dependent decrease in cell viability was accompanied by changes in cell morphology. Deformation in cell structure together with cell death revealed dose-dependent changes. Cell viability, which was 72% at a

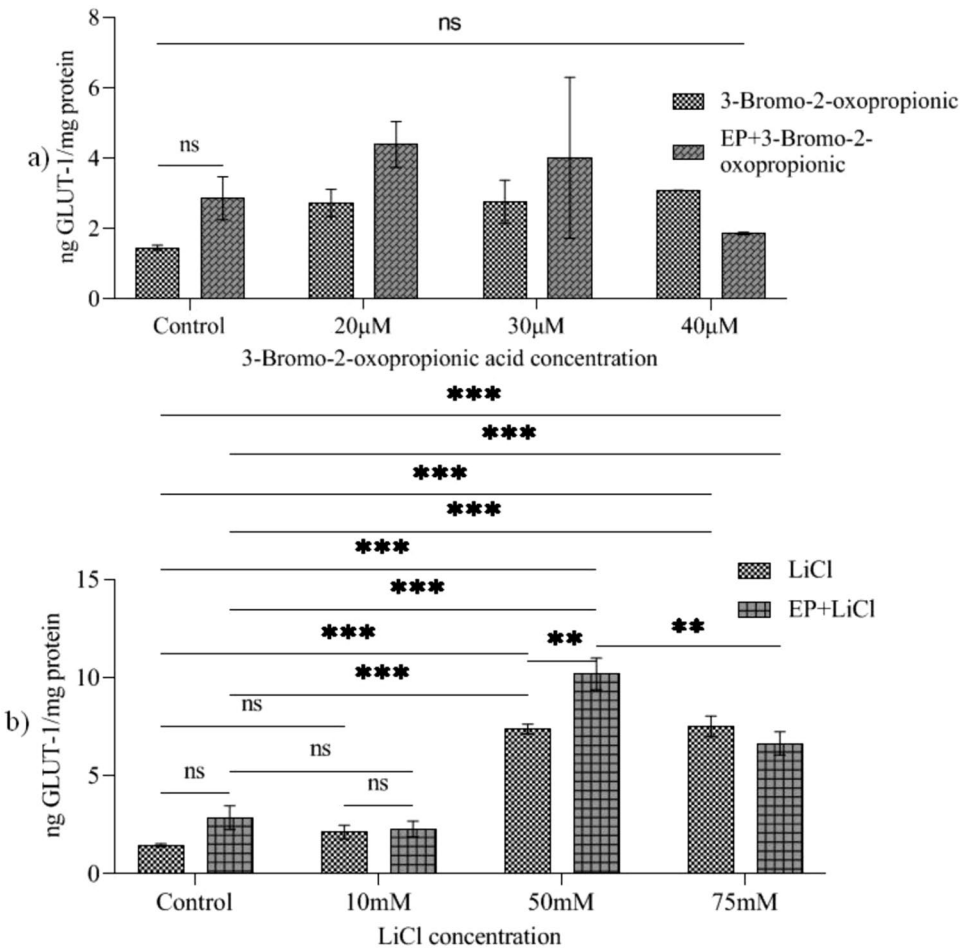

Fig. 7 Effects of 3-Bromo-2-oxopropionic acid and LiCl treatments alone and in combination with electroporation on GLUT-1 levels (ns: not significant, $*p<0.05$, $**p<0.01$ and $***p<0.001$)

concentration of 30 µM alone, showed a significant decrease of up to 50% in the combination with EP. In a study conducted on human colon carcinoma SW480 and HT29 cells using 3-Bromopyruvate (3BP), researchers determined a decrease in cell viability at increasing doses of 3BP from 0 to 320 µmol/L [18]. In another study conducted by Attia et al. on human breast cancer cells, 0–50 µM concentrations of 3BP were used and the IC_{50} value was determined as 36 µM [58]. In our study, the IC_{50} value of 3-Bromo-2-oxopropionic acid in DLD-1 human colon cancer cells was found to be 37.99 µM. In the combined treatment with EP, the IC_{50} value was found to be 27.33 µM. In the study conducted by Ho et al. on different human colon cancer cell lines, the IC_{50} values after 72 h of incubation were found to be 36.6 ± 2.1 for CaCo2 and 16.9 ± 1.3 µM for DLD-1 [59]. In another study conducted on human colorectal adenocarcinoma (HT-29) cells, researchers determined the IC_{50} dose as 120 µM. In the same study, researchers reported a significant increase in caspase-3 activity at the doses used for 3BR-P [60]. The fact that there was no significant change in caspase-3 activity in this study suggests that it may be related to the use of lower doses. However, combined applications with EP show a significant increase in caspase-3 compared to applications alone. Kielbik et al. reported that caspase-3 fluorescence signals were higher in 1000 and 1200 V/cm EP applications than in low electric fields [61]. The fact that caspase-3 activity we found in the study did not show significant increases compared to the control group suggests that cell death cannot be explained by classical apoptotic mechanisms and that alternative death pathways may have been involved. Indeed, in a study conducted with different colon cancer cell lines, it was revealed that 3BP can use different death mechanisms such as apoptosis, necroptosis and autophagy [18]. Similarly, in a study conducted by Mu et al. with different colon cancer cell lines, it was reported that 3BP induced autophagy-dependent ferroptosis [62]. In this respect, the study results support that 3-Bromo-2-oxopropionic acid may select cell death pathways different from apoptosis reported in the literature in its anti-cancer mechanism.

Changes in PI3K levels were investigated by applying 3-Bromo-2-oxopropionic acid alone and in combination with EP at concentrations of 20, 30 and 40 µM. While no significant change was detected in PI3K levels for all doses applied alone, it was found that PI3K levels increased with increasing doses in combined treatment with EP. In our literature

search for studies in combination with EP in cell lines, we could not find any sources regarding changes in PI3K levels. Therefore, this study is the first study to determine changes in PI3K levels in combination with EP. Considering the anticancer effects of 3-Bromo-2-oxopropionic acid, the general opinion is that PI3K levels tend to decrease [63]. However, in this study, an increase in PI3K levels was detected at concentrations of 30 and 40 µM combined with EP. On the other hand, Ho et al. reported that the expression of phosphoinositol-dependent protein kinase 1 (PDK1) increased in a dose-dependent manner in SW480 and DLD-1 cells [59]. Phosphoinositol-dependent protein kinase 1 (PDK1) plays a role in regulating intracellular functions from cell growth to proliferation, survival to differentiation, and has a hierarchical relationship in cell signaling, leading to the activation of the PI3K signaling pathway [64, 65]. Scheid et al. previously demonstrated that the change in the molecular weight of PDK1 is a result of hyperphosphorylation in response to a survival signal [66]. In addition, only the changes in total PI3K protein levels are expressed in this study, and it should be taken into account that the actual pathway activation should be evaluated by phosphorylation/dephosphorylation status. However, despite this, the study conducted by Ecker et al. Contrary to the current understanding that PI3K/AKT signaling pathway has a proliferative role, it has been reported that acute activation causes ROS-mediated cell death. ROS accumulation in the cell plays an important role in mediating different types of cell death [67, 68]. It has been reported that the PI3K/AKT pathway can be temporarily activated under chemotherapeutic stress conditions with compensatory cellular response mechanisms that can develop against anticancer agents and that this may be a metabolic adaptive response related to the survival effort of the cells [69].

In mammalian cells, glucose is the primary source of ATP production via glycolysis and oxidative phosphorylation. The transport of glucose from the plasma membrane to the cytosol is a rate-limiting step in glucose metabolism and is mediated by glucose transporter proteins (GLUTs) [70–72]. Glycolysis begins with the phosphorylation of glucose via the enzyme hexokinase (HK) and ends with the production of pyruvate, NADH and ATP as a result of a series of biochemical reactions. Normal cells maximize energy production by converting pyruvate to Acetyl-CoA in the presence of oxygen using the Krebs cycle and oxidative phosphorylation (OXPHOS) mechanisms [73, 74]. Cancer cells are heavily dependent on glycolysis to meet their energy needs, preferring glucose fermentation over mitochondrial oxidation despite the presence of oxygen [75]. In this study, no significant changes were detected in GLUT-1 levels in the applications of 3-Bromo-2-oxopropionic acid alone. GLUT-1 levels increased slightly in the applications of EP alone and combined, but these increases were not significant. In DLD-1 cells, 3-Bromo-2-oxopropionic acid alone and EP combined applications revealed an irregular change in GLUT-1 levels. GLUT-1, a transmembrane protein that enables the passive transport of glucose in cells, is expressed in many cell types. Due to its high glucose affinity, it plays a critical role in tissues where glucose is the primary energy source. GLUT-1 has been reported to be overexpressed in various types of cancer, including colorectal carcinoma, pancreatic and lung cancers [76–78].

It was determined that increasing doses of LiCl caused a dose-dependent decrease in cell viability in the DLD-1 colon cancer cell line. While cell viability was around 95% at 10 mM LiCl concentration, it was determined that a decrease of up to 75% occurred when combined with EP. The concentration value that inhibited half of DLD-1 cells was determined as 98.82 ± 0.64 mM when applied alone, while it was determined as 81.88 ± 1.64 mM when combined with EP. It was found that EP caused a significant decrease in LiCl dose. Li et al. reported that a dose-dependent decrease in viability occurred at different incubation times in their study on SW480 colon cancer cells [79]. Researches conducted with different doses of LiCl on different cancer cells have focused on providing a dose-dependent decrease in viability and explaining the death mechanisms [80–82].

Caspase-3 activity was examined in LiCl alone and in combination with EP for three different doses. Caspase-3 activity was increased dose-dependently in LiCl alone. In addition, the increase in caspase-3 activity was more pronounced in its combination with EP. It was determined that there was a statistically significant increase in caspase-3 activity compared to the control groups for the 75 mM LiCl dose. Studies have reported that LiCl up-regulates the release of factors such as apoptosis-inducing factor, cytochrome-c, and induces apoptosis by DNA fragmentation for many types of cancer including colon, pancreas, and leukemia [33, 34, 79]. Wu et al. demonstrated that treatment with LiCl in pancreatic cancer cells induced apoptosis by dose-dependent upregulation of BAX and caspase-3 and arrested the cell cycle in the G2/M phase [31]. Another examination conducted on MCF-7 cells showed that low doses of LiCl activated anti-apoptotic proteins, while high concentrations (50–100 mM) reversed these effects and induced apoptosis [83].

In the present study, it was determined that 10 mM LiCl application caused an increase in PI3K levels in DLD-1 cells. It was determined that the application of a relatively higher dose of 75 mM LiCl did not show a significant change in PI3K levels compared to the control group. A dramatic decrease was observed when the increase in PI3K levels occurred at 10 mM LiCl was compared to the 75 mM LiCl concentration combined with EP. In the study conducted by Suganthi et al., it was stated that LiCl gradually decreased Bax expression and increased Bcl-2 expression at a dose of

10 mM. The fact that LiCl applied at low doses suppressed p53 and Bax expressions while increasing Bcl-2 expression and Akt activity and provided cellular protection against apoptosis caused by glutamate excitotoxicity is supported by the present findings [83, 84].

In this study, the effects of three different doses of LiCl treatment and combined treatments with EP on GLUT-1 levels in DLD-1 cells were determined. While no significant change was determined in GLUT-1 levels for low LiCl dose alone and in combined treatments with EP, it was determined that GLUT-1 levels increased for 50 and 75 mM LiCl concentrations. It is known that the increase in GLUT-1 expression in cancer cells is related to cell survival. The increase in caspase-3 as well as the increase in GLUT-1 made the situation striking. However, it is also known that the increase in Reactive Oxygen Species (ROS) and oxidative stress is triggered as a result of the overexpression of glucose and oxidative phosphorylation [85]. ROS play an important role in determining the critical processes of cell differentiation or survival and are tightly regulated. However, it is stated that excessive ROS production in situations such as metabolic stress can trigger death mechanisms in the cell. High oxidative stress can increase DNA damage in the cell, which can lead to apoptosis. Metabolic changes that begin with an increase in GLUT-1 can, on the one hand, encourage the cell to grow, while on the other hand, it can bring the cell to the point of death [86, 87].

Conclusion

In this study, apoptotic activity and metabolic responses of 3-Bromo-2-oxopropionic acid and LiCl alone and in combination with EP were investigated on DLD-1 colon cancer cells. 3-Bromo-2-oxopropionic acid caused a dose-dependent decrease in cell viability, while this effect became more pronounced in combination with EP. In the treatment with the combination of both 3-Bromo-2-oxopropionic acid and LiCl with EP, a dramatic decrease in IC_{50} doses is observed. We believe that this will make a significant contribution to eliminating possible side effects of the treatment by allowing the use of lower drug doses in the combined treatment. While no significant change was observed in caspase-3 activity in alone applications, caspase-3 activity increased significantly in combination with EP. While no significant change was observed in PI3K levels in alone applications of 3-Bromo-2-oxopropionic acid, an increase was detected in combined applications with EP. Similarly, LiCl also decreased cell viability, while this effect became more pronounced in combination with EP. While caspase-3 activity increased at high concentrations in alone applications of LiCl, this increase was more pronounced when applied together with EP. In addition, while an increase was observed in PI3K levels at certain doses, no significant change was detected at higher doses. In terms of GLUT-1 levels, no significant change was observed at low doses, while an increase was recorded at higher doses. In conclusion, this study reveals that 3-Bromo-2-oxopropionic acid and LiCl cause changes in apoptotic mechanisms and metabolic responses and the effect of their combination with EP on these changes. In terms of metabolic responses, changes in PI3K and GLUT-1 levels provide important clues in understanding the metabolic adaptations of cells and their responses to treatment. In this context, it is thought that treatments combined with EP may have stronger anti-tumor effects in cancer cells, and it is recommended that these combinations be investigated in more detail in in vivo models and clinical studies.

Acknowledgements The authors would like to thank Prof. Dr. Ongun Onaran, Assoc. Prof. Dr. Meriç Arda Eşmekaya, Prof. Dr. Mehmet Özler and their team for their technical assistance.

Author contributions G. G. and Z. Ç. participated in the study design and coordination, conducted experimental studies and prepared the manuscript. All authors have read and approved the last manuscript.

Funding The authors declared that this study has received no financial support.

Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

References

1. Xi Y, Xu P. Global colorectal cancer burden in 2020 and projections to 2040. Transl Oncol. 2021;14(10):101174.
2. Mármol I, et al. Colorectal carcinoma: a general overview and future perspectives in colorectal cancer. Int J Mol Sci. 2017;18(1):197.
3. de Leon MP, Di Gregorio C. Pathology of colorectal cancer. Dig Liver Dis. 2001;33(4):372–88.
4. Chen W, et al. Drug delivery systems for colorectal cancer chemotherapy. Chin Chem Lett. 2023;35:109159.
5. Warburg O. On the origin of cancer cells. Science. 1956;123(3191):309–14.
6. Mathupala SP, Ko YH, Pedersen PL. Hexokinase II: cancer's double-edged sword acting as both facilitator and gatekeeper of malignancy when bound to mitochondria. Oncogene. 2006;25(34):4777–86.
7. Mathupala SP, Rempel A, Pedersen PL. Glucose catabolism in cancer cells. Isolation, sequence, and activity of the promoter for type II hexokinase. J Biol Chem. 1995;270(28):16918.
8. Pelicano H, et al. Glycolysis inhibition for anticancer treatment. Oncogene. 2006;25(34):4633–46.
9. Ciscato F, et al. Hexokinase 2 in cancer: a prima donna playing multiple characters. Int J Mol Sci. 2021;22(9):4716.
10. Palmieri D, et al. Analyses of resected human brain metastases of breast cancer reveal the association between up-regulation of hexokinase 2 and poor prognosis. Mol Cancer Res. 2009;7(9):1438–45.

11. Gottlob K, et al. Inhibition of early apoptotic events by Akt/PKB is dependent on the first committed step of glycolysis and mitochondrial hexokinase. Genes Dev. 2001;15(11):1406–18.
12. Katagiri M, et al. Hexokinase 2 in colorectal cancer: a potent prognostic factor associated with glycolysis, proliferation and migration. Histol Histopathol. 2017;32(4):351–60.
13. Nakajima K, et al. Glycolytic enzyme hexokinase II is a putative therapeutic target in B-cell malignant lymphoma. Exp Hematol. 2019;78:46-55.e3.
14. Guo D, et al. Hexokinases in cancer and other pathologies. Cell Insight. 2023;2(1):100077.
15. Cardaci S, Desideri E, Ciriolo MR. Targeting aerobic glycolysis: 3-bromopyruvate as a promising anticancer drug. J Bioenerg Biomembr. 2012;44(1):17–29.
16. da Silva WS, et al. Mitochondrial bound hexokinase activity as a preventive antioxidant defense: steady-state ADP formation as a regulatory mechanism of membrane potential and reactive oxygen species generation in mitochondria. J Biol Chem. 2004;279(38):39846–55.
17. El Sayed SM, et al. The promising anticancer drug 3-bromopyruvate is metabolized through glutathione conjugation which affects chemoresistance and clinical practice: an evidence-based view. Med Hypotheses. 2017;100:67–77.
18. Sun Y, et al. Mechanisms underlying 3-bromopyruvate-induced cell death in colon cancer. J Bioenerg Biomembr. 2015;47(4):319–29.
19. Ganapathy-Kanniappan S, et al. 3-Bromopyruvate induces endoplasmic reticulum stress, overcomes autophagy and causes apoptosis in human HCC cell lines. Anticancer Res. 2010;30(3):923–35.
20. El Sayed SM. Enhancing anticancer effects, decreasing risks and solving practical problems facing 3-bromopyruvate in clinical oncology: 10 years of research experience. Int J Nanomed. 2018;13:4699–709.
21. Azevedo-Silva J, et al. The anticancer agent 3-bromopyruvate: a simple but powerful molecule taken from the lab to the bedside. J Bioenerg Biomembr. 2016;48(4):349–62.
22. Ko YH, et al. Advanced cancers: eradication in all cases using 3-bromopyruvate therapy to deplete ATP. Biochem Biophys Res Commun. 2004;324(1):269–75.
23. Ko YH, et al. 3-Bromopyruvate as a potent anticancer therapy in honor and memory of the late Professor André Goffeau. Yeast. 2019;36(4):211–21.
24. Zhang Q, et al. Hexokinase II inhibitor, 3-BrPA induced autophagy by stimulating ROS formation in human breast cancer cells. Genes Cancer. 2014;5(3–4):100–12.
25. Berridge MJ, Downes CP, Hanley MR. Neural and developmental actions of lithium: a unifying hypothesis. Cell. 1989;59(3):411–9.
26. Campbell IH, Campbell H, Smith DJ. Insulin signaling as a therapeutic mechanism of lithium in bipolar disorder. Transl Psychiatr. 2022;12(1):350.
27. Duda P, et al. Targeting GSK3 and associated signaling pathways ınvolved in cancer. Cells. 2020;9(5):110.
28. Snitow ME, Bhansali RS, Klein PS. Lithium and therapeutic targeting of GSK-3. Cells. 2021. https://doi.org/10.3390/cells10020255.
29. Villegas-Vázquez EY, et al. Lithium: a promising anticancer agent. Life. 2023. https://doi.org/10.3390/life13020537.
30. Ngok-Ngam P, et al. Pharmacological inhibition of GSK3 attenuates DNA damage-induced apoptosis via reduction of p53 mitochondrial translocation and Bax oligomerization in neuroblastoma SH-SY5Y cells. Cell Mol Biol Lett. 2013;18(1):58–74.
31. Wu H, et al. Lithium chloride induces apoptosis by activating endoplasmic reticulum stress in pancreatic cancer. Transl Oncol. 2023;38:101792.
32. Yang C, et al. Lithium in cancer therapy: friend or foe? Cancers. 2023. https://doi.org/10.3390/cancers15041095.
33. Li L, et al. Lithium chloride promotes apoptosis in human leukemia NB4 cells by inhibiting glycogen synthase kinase-3 beta. Int J Med Sci. 2015;12(10):805–10.
34. Peng Z, et al. Lithium inhibits tumorigenic potential of PDA cells through targeting hedgehog-GLI signaling pathway. PLoS ONE. 2013;8(4):e61457.
35. Yang C, et al. Lithium in cancer therapy: friend or foe? Cancers (Basel). 2023;15(4):1095.
36. Bijelic L, Ramos I, Goeré D. The landmark series: surgical treatment of colorectal cancer peritoneal metastases. Ann Surg Oncol. 2021;28(8):4140–50.
37. Akhtar-Danesh N, et al. Uptake of minimally invasive surgery for early stage colorectal cancer and its effect on survival: a population-based study. Surg Oncol. 2020;35:540–6.
38. Lange MM, van de Velde CJ. Urinary and sexual dysfunction after rectal cancer treatment. Nat Rev Urol. 2011;8(1):51–7.
39. Gill S, Goldberg RM. First-line treatment strategies to improve survival in patients with advanced colorectal cancer. Drugs. 2004;64(1):27–44.
40. Leichman L. Neoadjuvant chemotherapy for disseminated colorectal cancer: changing the paradigm. J Clin Oncol. 2006;24(24):3817–8.
41. Jaroszeski MJ, et al. In vivo gene delivery by electroporation. Adv Drug Deliv Rev. 1999;35(1):131–7.
42. He H, Chang DC, Lee YK. Micro pulsed radio-frequency electroporation chips. Bioelectrochemistry. 2006;68(1):89–97.
43. Tung L, et al. Effects of strong electrical shock on cardiac muscle tissue. Ann NY Acad Sci. 1994;720:160–75.
44. Teissie J, Golzio M, Rols MP. Mechanisms of cell membrane electropermeabilization: a minireview of our present (lack of?) knowledge. Biochim Biophys Acta. 2005;1724(3):270–80.
45. Zimmermann U. Electric field-mediated fusion and related electrical phenomena. Biochim Biophys Acta. 1982;694(3):227–77.
46. Teissié J, Rols MP. An experimental evaluation of the critical potential difference inducing cell membrane electropermeabilization. Biophys J. 1993;65(1):409–13.
47. Kanduser M and Miklavcic D. Electroporation in biological cell and tissue: an overview. 2009, pp 1–37
48. Neumann E, Kakorin S, Toensing K. Fundamentals of electroporative delivery of drugs and genes. Bioelectrochem Bioenerg. 1999;48(1):3–16.
49. Mir LM, et al. Electrochemotherapy potentiation of antitumour effect of bleomycin by local electric pulses. Eur J Cancer. 1991;27(1):68–72.
50. Miklavčič D, et al. Electrochemotherapy: from the drawing board into medical practice. Biomed Eng Online. 2014;13(1):29.
51. Gursoy G, Esmekaya MA, Cicek Z. Treatment of cervical cancer by electrochemotherapy with bleomycin, cisplatin, and calcium: an in vitro experimental study. Med Oncol. 2022;40(1):52.
52. Poddevin B, et al. Very high cytotoxicity of bleomycin introduced into the cytosol of cells in culture. Biochem Pharmacol. 1991;42(Suppl):S67-75.
53. Pron G, et al. Internalisation of the bleomycin molecules responsible for bleomycin toxicity: a receptor-mediated endocytosis mechanism. Biochem Pharmacol. 1999;57(1):45–56.
54. Fyfe AJ, McKay P. Toxicities associated with bleomycin. J R Coll Physicians Edinb. 2010;40(3):213–5.
55. Gehl J, Skovsgaard T, Mir LM. Enhancement of cytotoxicity by electropermeabilization: an improved method for screening drugs. Anticancer Drugs. 1998;9(4):319–25.
56. Romeo S, et al. ESOPE-equivalent pulsing protocols for calcium electroporation: an in vitro optimization study on 2 cancer cell models. Technol Cancer Res Treat. 2018;17:1533033818788072.
57. Marty M, et al. Electrochemotherapy – an easy, highly effective and safe treatment of cutaneous and subcutaneous metastases: results of ESOPE (European standard operating procedures of electrochemotherapy) study. Eur J Cancer Suppl. 2006;4(11):3–13.

58. Attia YM, et al. Targeting glycolysis by 3-bromopyruvate improves tamoxifen cytotoxicity of breast cancer cell lines. BMC Cancer. 2015;15:838.
59. Ho N, et al. The effect of 3-bromopyruvate on human colorectal cancer cells is dependent on glucose concentration but not hexokinase II expression. Biosci Rep. 2016;36(1):e00299.
60. Nikravesh H, et al. The combined effect of dichloroacetate and 3-bromopyruvate on glucose metabolism in colorectal cancer cell line, HT-29; the mitochondrial pathway apoptosis. BMC Cancer. 2021;21(1):903.
61. Kiełbik A, et al. In vitro study of calcium microsecond electroporation of prostate adenocarcinoma cells. Molecules. 2020;25(22):5406.
62. Mu M, et al. 3-Bromopyruvate overcomes cetuximab resistance in human colorectal cancer cells by inducing autophagy-dependent ferroptosis. Cancer Gene Ther. 2023;30(10):1414–25.
63. Fan T, et al. Tumor energy metabolism and potential of 3-bromopyruvate as an ınhibitor of aerobic glycolysis: ımplications in tumor treatment. Cancers (Basel). 2019;11(3):317.
64. Barile E, De SK, Pellecchia M. PDK1 inhibitors. Pharm Pat Anal. 2012;1(2):145–63.
65. Vanhaesebroeck B, Alessi DR. The PI3K-PDK1 connection: more than just a road to PKB. Biochem J. 2000;346:561–76.
66. Scheid MP, Parsons M, Woodgett JR. Phosphoinositide-dependent phosphorylation of PDK1 regulates nuclear translocation. Mol Cell Biol. 2005;25(6):2347–63.
67. Redza-Dutordoir M, Averill-Bates DA. Activation of apoptosis signalling pathways by reactive oxygen species. Biochim Biophys Acta. 2016;1863(12):2977–92.
68. Ecker V, et al. Targeted PI3K/AKT-hyperactivation induces cell death in chronic lymphocytic leukemia. Nat Commun. 2021;12(1):3526.
69. Xia P, Xu XY. PI3K/Akt/mTOR signaling pathway in cancer stem cells: from basic research to clinical application. Am J Cancer Res. 2015;5(5):1602–9.
70. Adekola K, Rosen ST, Shanmugam M. Glucose transporters in cancer metabolism. Curr Opin Oncol. 2012;24(6):650–4.
71. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science. 2009;324(5930):1029–33.
72. Medina RA, Owen GI. Glucose transporters: expression, regulation and cancer. Biol Res. 2002;35(1):9–26.
73. Zambrano A, et al. Glut 1 in cancer cells and the ınhibitory action of resveratrol as a potential therapeutic strategy. Int J Mol Sci. 2019;20(13):3374.
74. Rolfe DF, Brown GC. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. Physiol Rev. 1997;77(3):731–58.
75. Warburg O, Wind F, Negelein E. The metabolism of tumors in the body. J Gen Physiol. 1927;8(6):519–30.
76. Joost HG, Thorens B. The extended GLUT-family of sugar/polyol transport facilitators: nomenclature, sequence characteristics, and potential function of its novel members (review). Mol Membr Biol. 2001;18(4):247–56.
77. Jun YJ, et al. Clinicopathologic significance of GLUT1 expression and its correlation with Apaf-1 in colorectal adenocarcinomas. World J Gastroenterol. 2011;17(14):1866–73.
78. Sakashita M, et al. Glut1 expression in T1 and T2 stage colorectal carcinomas: its relationship to clinicopathological features. Eur J Cancer. 2001;37(2):204–9.
79. Li H, et al. Lithium chloride suppresses colorectal cancer cell survival and proliferation through ROS/GSK-3β/NF-κB signaling pathway. Oxid Med Cell Longev. 2014;2014:241864.
80. Erguven M, et al. Lithium chloride has a biphasic effect on prostate cancer stem cells and a proportional effect on midkine levels. Oncol Lett. 2016;12(4):2948–55.
81. Hou L, et al. Lithium protects dopaminergic cells from rotenone toxicity via autophagy enhancement. BMC Neurosci. 2015;16(1):82.
82. Suganthi M, et al. In vitro mechanisms involved in the regulation of cell survival by lithium chloride and IGF-1 in human hormone-dependent breast cancer cells (MCF-7). Toxicol Lett. 2012;214(2):182–91.
83. Suganthi M, et al. Biphasic dose-dependent effect of lithium chloride on survival of human hormone-dependent breast cancer cells (MCF-7). Biol Trace Elem Res. 2012;150(1–3):477–86.
84. Chen RW, Chuang DM. Long term lithium treatment suppresses p53 and Bax expression but increases Bcl-2 expression. A prominent role in neuroprotection against excitotoxicity. J Biol Chem. 1999;274(10):6039–42.
85. Burgos-Morón E, et al. Relationship between oxidative stress, ER stress, and ınflammation in type 2 diabetes: the battle continues. J Clin Med. 2019;8(9):1385.
86. Villalpando-Rodriguez GE, Gibson SB. Reactive oxygen species (ROS) regulates different types of cell death by acting as a rheostat. Oxid Med Cell Longev. 2021;2021:9912436.
87. Rakıcıoğlu N, Çetiner Ö. Hiperglisemi, oksidatif stres ve tip 2 diyabette oksidatif stres belirteçlerinin tanimlanması. Turkish J Diabetes Obes. 2020;4(1):60–8.

Authors and Affiliations

Güney Gürsoy[1] · **Zehra Çiçek**[2]

✉ Güney Gürsoy
guneygursoy@ahievran.edu.tr

[1] Department of Biophysics, Department of Basic Medical Sciences, Faculty of Medicine, Kırşehir Ahi Evran University, Kırşehir, Turkey

[2] Department of Physiology, Gülhane Faculty of Medicine, University of Health Sciences, Ankara, Turkey