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RESEARCH ARTICLE

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Lithium salts cytotoxicity and accumulation in melanoma cells in vitro

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Abstract

Boron neutron capture therapy is a perspective selective technology for the destruction of cancer cells, while the use of lithium instead of boron may represent a new and promising vector for the development of neutron capture therapy (NCT). The aim of the study was a comparative assessment of the cytotoxicity of various lithium salts, as well as an analysis of the accumulation of lithium in tumor cells in vitro to determine the possibility of using lithium in NCT. The cytotoxicity of lithium salts was determined using MTT-test and colony forming assay on human fibroblasts BJ-5ta, human skin melanoma SK-Mel-28, and mouse skin melanoma B16 cell lines. An assessment of lithium concentration in cells was performed using inductively coupled plasma atomic emission spectrometry. Our results showed that three different lithium salts at a concentration of 40 μg/ml are not toxic for both tumor and normal cells. The highest uptake values were obtained on murine melanoma B16 cells when exposed to lithium carbonate (0.8 μg/10^6 cells); however, human melanoma SK-Mel-28 cells effectively accumulated both lithium carbonate and lithium citrate (about 0.46 μg/10^6 cells for two salts). Thus, our results demonstrate a range of non-toxic doses of lithium salts and a high uptake of lithium by tumor cells, which indicates the possibility to use the lithium in NCT.

KEYWORDS
cytotoxicity, inductively coupled plasma atomic emission spectrometry, lithium salts, neutron capture therapy, skin melanoma

1 | INTRODUCTION

Boron neutron capture therapy (BNCT) is a type of radiation therapy based on the boron neutron capture nuclear reaction ($^{10}B[n,\alpha]^7Li$) (Suzuki, 2020). BNCT is a selective method for the destruction of cancer cells, since ^{10}B atoms, after absorbing thermal neutrons, decay into an alpha particle and a lithium nucleus and release most of the nuclear reaction energy within one cell (about 10 μm) (Matsumoto et al., 2021; Suzuki, 2020). Locher (1936) proposed the concept of neutron capture therapy in the mid-1930s, but only after the end of the Second World War, the development of nuclear reactors for medicine allowed to start clinical trials of BNCT (Jin et al., 2022). Although the clinical trials showed the promising results, there is a number of limitations to the widespread use of BNCT technology (Cheng et al., 2022; Jin et al., 2022). Achieving optimal boron concentrations in tumor cells, normal tissue, and blood is the most important problem in the BNCT (Cheng et al., 2022; Sauerwein et al., 2021). Currently, despite the development of new targeted boron delivery drugs, only second-generation boron drugs, such as boronophenylalanine and sodium borocaptate, are being used in clinical trials (Barth et al., 2018; Dymova et al., 2020).

The use of lithium instead of boron may represent a new and promising vector for the development of NCT. Lithium has a number of physical characteristics that can provide large thermal neutron absorption cross-section (940 b) (Sauerwein et al., 2012) and 100%

local energy release within the cell due to high linear energy transfer of reaction products. The use of most isotopes with thermal neutron absorption cross-section more than 500 b (such as ^{113}Cd, ^{135}Xe, ^{149}Sm, ^{151}Eu, and Gd), unlike lithium, will lead to (n,γ)-reaction and the absence of local energy release.

The history of lithium salts application in medicine dates back to the middle of the 20th century, when Cade (1949) showed the lithium effects for mania treatment in psychiatric patients. However, the high toxicity of lithium significantly slowed down its widespread use in medical practice, until the 1970s when the US Food and Drug Administration agency approved the use of lithium for the treatment of mania (Mitchell & Hadzi-Pavlovic, 2000). Currently, the most commonly used class of medications for bipolar disorders is lithium salts, such as lithium carbonate, lithium chloride, lithium citrate, and lithium sulfate (Malhi et al., 2012; Oruch et al., 2014).

Lithium has a relatively narrow therapeutic index that may increase the probability of adverse drug reactions. However, the accumulated data about the lithium pharmacokinetics parameters allow to control of lithium concentrations to prevent the development of side effects and toxicity (Kakhki & Ahmadi-Soleimani, 2022; Wen et al., 2019). Thus, the aim of this work was a comparative assessment of the cytotoxicity of various lithium salts, as well as an analysis of the accumulation of lithium in tumor cells in vitro to determine the possibility of using lithium in neutron capture therapy.

2 | MATERIALS AND METHODS

2.1 | Cell lines

Human fibroblasts BJ-5ta and human skin melanoma SK-Mel-28 cells were obtained from the Center of Genetic Resources of Laboratory Animals, ICG SB RAS (RFMEFI62119X0023), and mouse skin melanoma B16 cells were obtained at the Institute of Cytology and Genetics SB RAS (Novosibirsk, Russia) (Figure 1A). Cells were incubated in DMEM/F12 (Biolot) with 10% fetal bovine serum (Biolot) and Gentamicin 50 mg/ml (Dalkhimpharm) at 37°C and 5% CO_2. Cells were subcultured using a trypsin–EDTA solution two to three times a week in a ratio of 1:3 or 1:5. The cells were involved in the experiment at the third passage.

2.2 | MTT test

Lithium carbonate (Li_2CO_3), lithium citrate ($C_6H_5Li_3O_7$), and lithium chloride (LiCl) were obtained from "Novosibirsk rare metals plant" (Russia). The cytotoxicity of lithium carbonate, citrate, and chloride was determined using MTT test. This colorimetric assay is based on the ability of mitochondrial enzymes of living cells to reduce the reagent into a colored formazan product soluble in dimethyl sulfoxide (DMSO). The decrease in optical density is inversely proportional to the number of metabolically active cells, which indicates the cytotoxic effect of the drug (Mosmann, 1983).

Cells were seeded in 96-well plates 4×10^4 cells per well and incubated for 24 h. Then, lithium drugs were added to the wells at lithium concentrations ranging from 10 to 640 μg/ml. The plates were incubated for 24 h. Wells with cells in the medium without the drug were taken as controls. Then, the medium with the drugs was replaced with a medium without serum, and 10 μl of MTT reagent at a concentration of 5 mg/ml was added to each well. The plates were incubated under standard conditions for 4 h, and then, the medium was replaced with DMSO. A Multiskan SkyHigh (Thermo Fisher Scientific, Waltham, MA, United States) microplate reader measured the absorbance at 595 nm. Cell viability was calculated using the values of optical density: viability (%) = average optical density in the experimental group/average optical density in the control group ×100%.

2.3 | Colony forming assay

The colony forming assay was used to evaluate the effect of lithium salts on proliferation capacity of cell lines. Cells were incubated in culture flasks; in the logarithmic phase of growth, the medium containing lithium salts at lithium concentration of 40 μg/ml was added, and the flasks were incubated under standard conditions for 24 h. Then, cells were washed with PBS (Biolot) and removed from the culture plates using a trypsin–EDTA solution (Biolot) and seeded at a density of 200 cells per well in six-well plates and were incubated for 10 days under standard conditions. Then, the plates were washed with phosphate buffer saline (PBS), fixed with 10% formalin (Panreac AppliChem, Darmstadt, Germany), stained with Giemsa solution (Sigma, St. Louis, MO, United States), and dried. The calculation was carried out using an inverted light microscope Zeiss Primo Vert (Oberkochen, Germany); the colonies of greater than 50 cells were counted (Franken et al., 2006).

2.4 | Inductively coupled plasma atomic emission spectrometry

Cells were incubated in culture flasks; in the logarithmic phase of growth, the medium was changed to the medium containing lithium salts at a lithium concentration of 40 μg/ml and cultured under standard conditions for 24 h. Then, the cells were washed with PBS and removed from the plastic using a trypsin–EDTA solution. Precipitate sample preparation was carried out in tubes with loose lid using concentrated nitric acid HNO_3 (high purity grade 27-5, Panreac AppliChem, Darmstadt, Germany) at temperature of 90°C in Dry Block Heater 2 (IKA, Königswinter, Germany) until the liquid became clear. The lithium concentration was measured by inductively coupled plasma atomic emission spectrometry (ICP AES) on an ICPE-9820 high-resolution spectrometer (Shimadzu, Kyoto, Japan). The device was calibrated using Lithium (Li) CRISTAR® 1,000 ppm Single Element Std. Soln. for ICP in HNO_3 (Central Drug House [P] Ltd, Delhi, India) in the range of 0.01–10 mg/L.

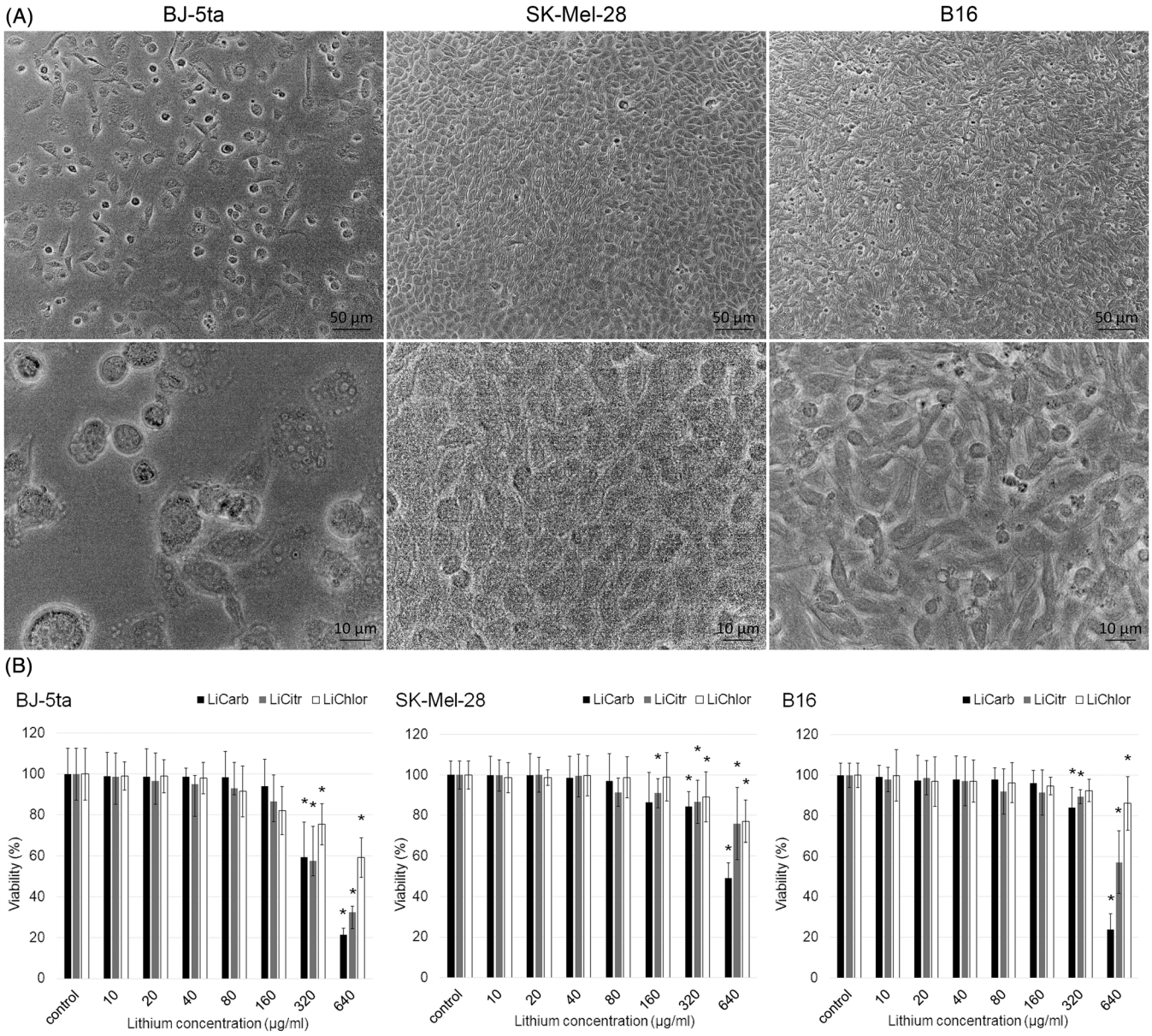

FIGURE 1 Morphology of BJ-5ta, SK-Mel-28, and B16 cell lines (A). An assessment of lithium salts cytotoxicity in a wide range of increasing Li concentrations on BJ-5ta, SK-Mel-28, and B16 cells in culture after 24 h by MTT test ($n = 5$) (B). *$p < 0.05$ compared with the control group. Data are presented as means ± SD. LiCarb, lithium carbonate; LiCitr, lithium citrate; LiChlor, lithium chloride

3 | RESULTS

3.1 | The cytotoxicity of lithium salts estimated by MTT test

Cytotoxicity of lithium salts in vitro was evaluated after incubation with the drugs for 24 h using MTT test (Figure 1B). Lithium carbonate did not show any toxic effect on all cell lines in the range of lithium concentrations of 10–160 µg/ml. Statistically significant differences in cell viability of experimental groups from the control groups were first obtained at a lithium concentration of 320 µg/ml ($p = 0.0209$ for BJ-5ta, $p = 0.004$ for SK-Mel-28, and $p = 0.0181$ for B16 cell lines). The most obvious cytotoxic effect was observed at the highest lithium concentration of 640 µg/ml; the survival of BJ-5ta, SK-Mel-28, B16 cells was 21%, 24%, and 49%, respectively.

There was no cytotoxic effect of lithium citrate in the range of lithium concentrations of 10–160 µg/ml observed on BJ-5ta and B16 cell lines; for the SK-Mel-28 cell line, a concentration of 160 µg/ml significantly reduced the percentage of viable cells compared to the intact control ($p = 0.0476$).

Similar results were obtained in lithium chloride-treated group: cell viability of three lines exposed to lithium in the concentration range of 10–160 µg/ml was not statistically different from the results obtained in the control groups. The viability of B16 cells decreased to 92.5% when exposed to lithium chloride in lithium concentration of 320 µg/ml, which did not significantly differ from the control.

However, lithium chloride at the same concentration of lithium has shown a cytotoxic effect on the other cell lines: the viability of BJ-5ta cells was 75.5%, and SK-Mel-28 cells was 89%.

3.2 | Evaluation of colony forming capacity of tumor and normal cell lines after 24 h exposure with lithium salts

Analysis of colony forming capacity of tumor and normal cell lines after incubation with lithium salts at lithium concentration of 40 μg/ml for 24 h was performed using colony forming assay (Figure 2). There were no statistically significant differences between survival fractions in the experimental and control groups.

3.3 | Assessment of lithium accumulation in cells by ICP AES

An assessment of lithium concentration in cells after incubation with lithium salts in lithium concentration of 40 μg/ml for 24 h was performed using ICP AES (Figure 3). Lithium concentration in BJ-5ta was highest for lithium carbonate and was 0.47 μg/10^6 cells. Lithium citrate and lithium chloride were accumulated less intensive, than lithium carbonate, and the concentrations of lithium were 0.12 and 0.18 μg/10^6 cells, respectively. Lithium average uptakes by SK-Mel-28 cell line were 0.46, 0.47, and 0.36 μg/10^6 cells when incubated with lithium carbonate, lithium citrate, and lithium chloride, respectively. The highest value (0.79 μg/10^6 cells) was determined in B16 cells incubated with lithium carbonate. Nevertheless, lithium accumulation was only 0.11 and 0.12 μg/10^6 cells in lithium citrate and lithium chloride groups.

4 | DISCUSSION

In this work, we suggest that lithium can be a good alternative to boron for NCT. To confirm this, first, it is necessary to assess the potential lithium toxicity at the concentrations effective for NCT and the lithium accumulation in tumor cells. According to theoretical calculations, lithium concentrations in the tumor should be 40 μg/g or more for a successful neutron capture reaction. It is generally

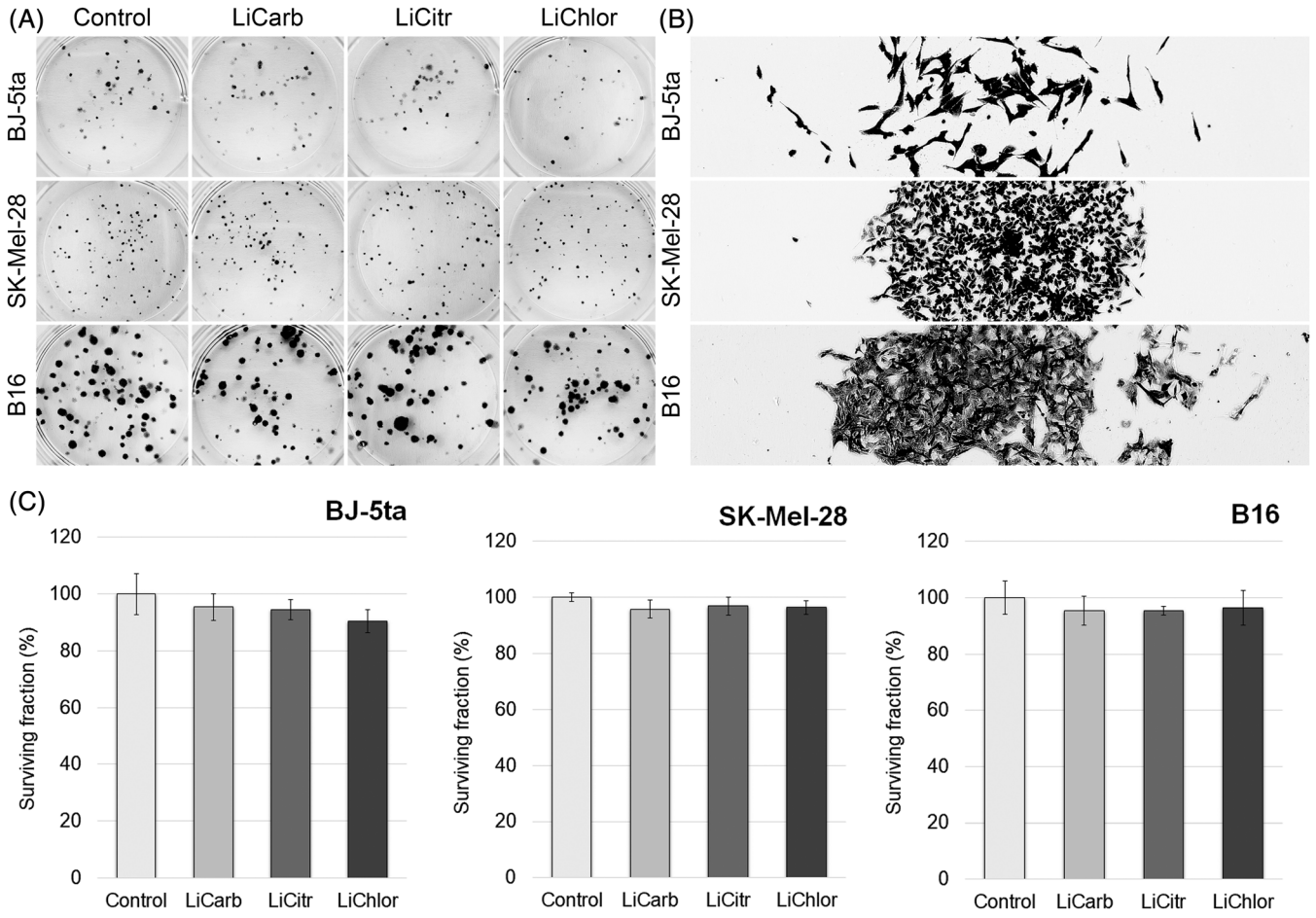

FIGURE 2 Evaluation of colony forming capacity of BJ-5ta, SK-Mel-28 и B16 cells 10 days after lithium salts exposure using colony forming assay ($n = 5$). Colony-formation ability of BJ-5ta, SK-Mel-28 и B16 cells lines (A). Representative images of the colony forming units, magnification 100X (B). Survival of different cell lines (C). Data are presented as means ± SD. LiCarb, lithium carbonate; LiCitr, lithium citrate; LiChlor, lithium chloride

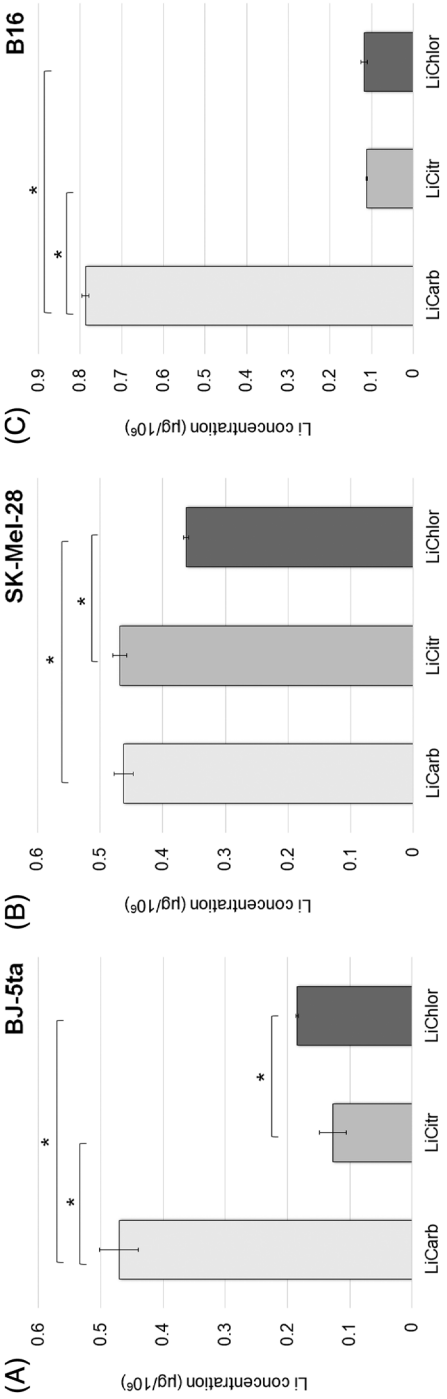

FIGURE 3 Lithium uptake by BJ-5ta, SK-Mel-28 and B16 cells after 24 h of incubation with lithium salts assessed by inductively coupled plasma atomic emission spectrometry ($n = 3$). Data are presented as means ± SD. LiCarb, lithium carbonate; LiCitr, lithium citrate; LiChlor, lithium chloride

accepted that boron concentration in the tumor is required 20 μg/g or more for BNCT reaction (Barth et al., 2018). In that case, since the cross-section of the $^{6}Li(n,\alpha)^{3}H$ reaction is four times smaller than the cross-section of the $^{10}B(n,\alpha)^{7}Li$ reaction (940 b instead of 3,835 b), and the energy released in the cell is two times higher (4.785 MeV instead of 84% from 2.79 MeV), the optimal lithium concentration should be ≥40 μg/g.

Our results showed that three different lithium salts at a concentration of 40 μg/ml are not toxic for both tumor and normal cells. An increase in the toxicity of lithium salts was observed when using lithium at a concentration of ≥160 μg/ml. Thus, our data demonstrate the possibility of using all three lithium salts at concentrations minimally required for successful NCT.

Since the accumulation of boron drugs in the tumor is one of the most important conditions for BNCT, we also decided to evaluate the accumulation of lithium in tumor and normal cells. In addition to the current use of lithium in the therapy of bipolar disorders, lithium is also being investigated as a drug for experimental cancer therapy. It was found that lithium has the various antitumor effects in cancer models in vitro and in vivo (Villegas-Vázquez et al., 2023; Yang et al., 2023); however, the data regarding lithium accumulation in tumors are limited. Previously, we have revealed that lithium carbonate accumulates in the tumor at a concentration higher than 20 μg/g in B16 mouse melanoma model in vivo (Taskaeva et al., 2023); nevertheless, it is not clear whether accumulation is the same for different melanoma cell lines or other cell lines. In addition, it is not known how tumor cells will absorb other lithium salts commonly used in medicine, such as lithium citrate and lithium chloride.

Clinical trials of BNCT had begun with the use of boric acids as a boron delivery agent (Wang et al., 2022). In 1968, encouraging results of BNCT were showed by Hatanaka et al. using borocaptate (Hatanaka, 1990; Hatanaka & Nakagawa, 1994) and in 1987 by Mishima et al. (1989) using boronophenylalanine (BPA). Despite the active development of the chemistry of boron-containing compounds, borocaptate and boronophenylalanine are still used in clinical trials of BNCT, although these drugs do not fully meet the requirements for boron-containing agents for BNCT (Barth et al., 2018; Novopashina et al., 2021; Wang et al., 2022).

When BPA was used at a concentration of 40 μg/ml after 24 h, boron concentrations in glioma cells were found to range from 0.8–1 μg/10^{7} cells (Yoshida et al., 2020). In a study of three melanoma cell lines incubated with BPA for 24 h at a concentration of 50 μg/ml, boron accumulation ranged from 0.04 to 0.12 μg/10^{6} cells, depending on the cell line (Carpano et al., 2015). Also, a combined short incubation of cells with BPA (1–3 h) at a concentration of 2 mM (418 μg/ml) showed boron uptake of 19 μg/10^{9} cells for mouse melanoma B16 and 13 μg/10^{9} cells by glioma cells (Yang et al., 2014), and at a concentration of 3 mM, boron uptake was about 1.5 μg/10^{7} cells for mouse melanoma and colon cancer cells (Tsurubuchi et al., 2020). Similar results were obtained when glioma cells were co-incubated with 1 mM borocaptate (0.86 μg/10^{7} cells) and 1 mM BPA (about 3.3 μg/10^{7} cells) (Futamura et al., 2017).

Our results revealed heterogeneous accumulation of lithium in vitro using lithium carbonate, lithium citrate, and lithium chloride. The highest uptake values were obtained on murine melanoma B16 cells when exposed to lithium carbonate (0.8 μg/10^6 cells); however, human melanoma SK-Mel-28 cells effectively accumulated both lithium carbonate and lithium citrate (about 0.46 μg/10^6 cells for two salts). Normal human fibroblasts also absorbed lithium carbonate quite well, with significantly lower accumulation of lithium citrate and lithium chloride. Thus, our results demonstrate significantly higher uptake of lithium by tumor cells compared to boron drugs approved for clinical use.

Despite the lithium is present in most body tissues, the kidney, thyroid, and brain are some of the organs with a physiologically higher intracellular than extracellular lithium concentration; thus, the main side effects from lithium poisoning can affect these organs (Baird-Gunning et al., 2017; McKnight et al., 2012; van Deun et al., 2021). Lithium carbonate is the most frequently used lithium salt for bipolar disorder treatment; however, lithium salts have different pharmacokinetic profile, and the absorption of various lithium salts by organs may differ. In particular, lithium orotate is proposed to cross the blood–brain barrier and enter cells more readily than lithium carbonate (Pacholko & Bekar, 2021). The "plateau effect" and modulated brain biodistribution was found after lithium salicylate administration that can be associated with absorption, distribution, metabolism, and/or elimination effects from the salicylate anion (Smith et al., 2014). Study of lithium ascorbate biodistribution indicated accumulation of lithium ions in a sort of "depot," consisting of the brain, aorta, and femur (Torshin et al., 2022).

Boron and its metabolites are excreted primarily by the urinary system, which explains the high concentrations of boron in the kidneys (Tang et al., 2022). Horn et al. (1997) revealed that a single infusion of sodium borocaptate can induce some alterations in the function of a normal kidney, resulting in a reduction of the filtration fraction. Increase in urine production in patients with brain tumors was observed after sodium borocaptate infusion, and the changes of glomerular filtration rate and urine flow rate were not significant (Horn et al., 1997). The toxic effects of L-10BPA have been associated with its low penetration of the blood–brain barrier (Roda et al., 2014). At the same time, the results of boron-related long-term toxicity trials were not published (Sauerwein et al., 2012). The data about toxic effects of boron delivery drugs on thyroid are limited.

It is known that intratumoral heterogeneity of melanoma cells affects the absorption and accumulation of boron (Rossini et al., 2015). In the line of this, we suggest that the data obtained in our study about lithium accumulation can be associated with tumor cells heterogeneity. Additionally, in this study, we used lithium salts as non-selective agents to deliver high concentrations of lithium to tumor cells. The concentrations used did not show cytotoxicity for both tumor and normal cells. The development of selective delivery agents containing lithium can allow achieving higher concentrations of lithium in the tumor. In addition, analysis of lithium accumulation in other cancer cell lines may help to evaluate the lithium uptake properties of different tumor cell types. Finally, further studies using isotope ^{6}Li are required for practical verification of the feasibility of lithium neutron capture therapy. It will be also interesting to understand whether there is a synergistic enhancement when using the drug with boron and lithium simultaneously.

AUTHOR CONTRIBUTIONS

Iuliia Taskaeva: Conceptualization; data analysis; writing and original draft; review and editing. **Anna Kasatova**: In vitro studies; data analysis; writing, review, and editing. **Ivan Razumov**: In vitro studies; data analysis; review and editing. **Nataliya Bgatova**: In vitro studies; data analysis; review and editing. **Sergey Taskaev**: Conceptualization; data analysis; review and editing. All authors have read and approved the final version of the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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