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Comparative Study of Cytotoxicity and Accumulation of Boron and Lithium-Containing Drugs in Skin Melanoma Cells *In Vitro*

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We studied cytotoxicity and accumulation of boron and lithium by cultured human fibroblasts and human and mouse skin melanoma cell cultures. The cytotoxicity of boron and lithium drugs was assessed by MTT tests in the boron and lithium concentration range of 10-640 μg/ml. Cell viability was significantly reduced after incubation with boron and lithium at concentrations >160 μg/ml. To assess accumulation of boron and lithium, the concentration of elements was measured using inductively coupled plasma atomic emission spectrometry. Melanoma cells more intensively accumulated lithium in comparison with boron. The results indicate the possibility of safe application of lithium salts in concentrations minimally required for successful neutron capture therapy.

Key Words: *boronophenylalanine; borocaptate; lithium salts; skin melanoma; neutron capture therapy*

The development of technologies for the selective destruction of tumor cells is a current trend in oncology, and one of such techniques is boron neutron capture therapy (BNCT). This technology is based on interaction of non-radioactive ^{10}B isotope with a thermal neutron; as a result of the nuclear reaction $^{10}B(n,\alpha)^{7}Li$ [1], 84% of the energy is released within one cell. Clinical trials of BNCT began with the use of boric acid and its salts as a boron delivery agent [2]. Currently, despite active development of the chemistry of boron-containing compounds, second-generation drugs, boronophenylalanine and sodium borocaptate, are commercially available and registered agents for clinical trials [3-5], although these drugs do not fully meet the requirements for boron-containing agents for BNCT [6,7]. High concentrations of boron in the tumor were obtained using the latest generation of boron drugs, which significantly increase the selectivity of its delivery to tumor cells (liposomes, polymers, nanoparticles, aptamers, *etc.*) in experiments *in vitro* and *in vivo* [8,9]; however, they have not been introduced into practice of clinical research.

The use of lithium instead of boron in neutron capture therapy (NCT) is a promising direction for the development of this technology. Lithium has a large absorption cross-section for thermal neutrons (940 barn) [1] and when interacting with a neutron, 100% local energy release occurs inside the cell due to the reaction products with high linear energy transfer. Successful boron-neutron capture reaction requires a boron concentration in the tumor of at least 20 μg/g [1,6]. According to theoretical calculations, the neutron absorption cross-section for ^{6}Li is 4 times smaller than for ^{10}B (940 b *vs* 3835 b), and the energy release in the $^{6}Li(n,\alpha)^{3}H$ reaction is 2 times higher than in the $^{10}B(n,\alpha)^{7}Li$ reaction (4.785 MeV *vs* 84% of 2.79 MeV), therefore, for a successful neutron capture reaction with lithium, the optimal lithium concentration in the

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tumor should be 40 μg/g or more. It is currently unknown how efficiently lithium will be taken up by tumor cells compared to boron. Previously, single studies on the ability of tumor cells to accumulate lithium have been conducted [10,11], but the ability of melanoma cells to uptake lithium is still unknown.

Thus, the aim of this work was to compare the cytotoxicity and accumulation of boron and lithium to determine the possibility of using lithium in NCT.

MATERIALS AND METHODS

The cytotoxicity of the drugs and the accumulation of boron and lithium *in vitro* were assessed using cell cultures BJ-5ta (normal human fibroblasts), B16 (mouse skin melanoma), and SK-Mel-28 (human skin melanoma) obtained at the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences.

The cytotoxicity of boron (boronophenylalanine and borocaptate, Katchem) and lithium (lithium carbonate and lithium citrate, Novosibirsk Rare Metals Plant) drugs was assessed by the MTT test (5 replicates for each experimental group). The cells were seeded in a 96-well plate (4×10^4 cells/well). Then boron and lithium drugs were added to the wells in the range of element concentrations in the solution from 10 to 640 μg/ml. Wells with cells containing medium without drugs were used as control groups. In 24 h, 10 μl of MTT solution at a concentration of 5 mg/ml and 100 μl of serum-free medium were added to each well. The optical density (OD) of solutions was measured on a Multiskan SkyHigh spectrophotometer (Thermo Fisher Scientific) at 595 nm. Cell survival was calculated by the formula:

$$OD_{exp}/OD_{control}\times100\%.$$

The concentration of boron and lithium in cells was measured by inductively coupled plasma atomic emission spectrometry (ICP AES). The cells were incubated in 25-μl flasks for 24 h, then the medium was changed to a medium containing boron or lithium drugs at a boron or lithium concentration of 40 μg/ml of culture medium, then the flasks were incubated under standard conditions for 24 h. Control cells were cultured without boron or lithium drugs. After incubation the cells were harvested and counted. The samples were prepared by wet ashing using concentrated nitric acid HNO_3 (chemically pure; Panreac AppliChem) at 90°C in a Dry Block Heater 2 heating system (IKA). The boron and lithium concentrations (3 replicates for each experimental group) were measured using ICPE-9820 high-resolution spectrometer (Shimadzu). Calibration dependences were constructed using a single-element standard solution of Boron Standard for ICP (Sigma-Aldrich) or Lithium Single Element Std. Soln. for ICP (Central Drug House (P) Ltd) in a range of 0.01-10 mg/liter. To obtain the final concentration of boron or lithium in cells, the formula was used: measured concentration×sample volume/number of cells$\times10^6$.

The data were processed statistically using Microsoft Excel and Statistica 6.0 software (StatSoft, Inc.). The results are presented as the mean (*M*) and standard deviation (*SD*). Significance of differences between the studied parameters was assessed by the Mann–Whitney *U* test (nonparametric statistics) at a confidence level of 95% ($p<0.05$).

RESULTS

The maximum non-toxic concentration of boron for BJ-5ta fibroblasts was 80 μg/ml (Fig. 1). Incubation of B16 mouse skin melanoma with borocaptate and boronophenylalanine did not significantly decrease cell survival in the boron concentration range up to 160 and 320 μg/ml, respectively. The cytotoxic effect of boron drugs on SK-Mel-28 was detected at a boron concentration of 640 μg/ml.

Lithium carbonate had no significant toxic effect on all cell lines in the lithium concentration range from 10 to 160 μg/ml. Lithium citrate also had no toxic effect on cell cultures BJ-5ta and B16 in the range of lithium concentrations up to 160 μg/ml, however, the survival of SK-Mel-28 cells after incubation with this drug at a lithium concentration of 160 μg/ml was significantly reduced, compared to the control group. Thus, the cytotoxicity of the studied lithium salts did not differ significantly from boron drugs.

The accumulation of boron and lithium in normal and tumor cells was measured using ICP AES (Fig. 2). In BJ-5ta cells incubated with boronophenylalanine and borocaptate, boron concentration was 0.23 and 0.24 μg/10^6 cells, respectively. The maximum boron concentration was achieved in SK-Mel-28 and B16 cells (0.29 μg/10^6 cells) incubated with boronophenylalanine. After incubation with borocaptate, the boron concentration was 0.21 μg/10^6 cells for the SK-Mel-28 culture and 0.19 μg/10^6 cells for B16. Lithium concentrations in BJ-5ta cells were 0.47 μg/10^6 cells after incubation with lithium carbonate, and 0.12 μg/10^6 cells after incubation with lithium citrate. The accumulation of lithium in SK-Mel-28 cells was 0.46 and 0.47 μg/10^6 cells after incubation with lithium carbonate and lithium citrate, respectively. The highest concentration of lithium was detected in B16 cells after incubation with lithium carbonate (0.79 μg/10^6 cells), while after incubation with lithium citrate, lithium accumulation was 0.11 μg/10^6 cells.

The obtained results on boron accumulation in tumor cells are consistent with the data of other studies:

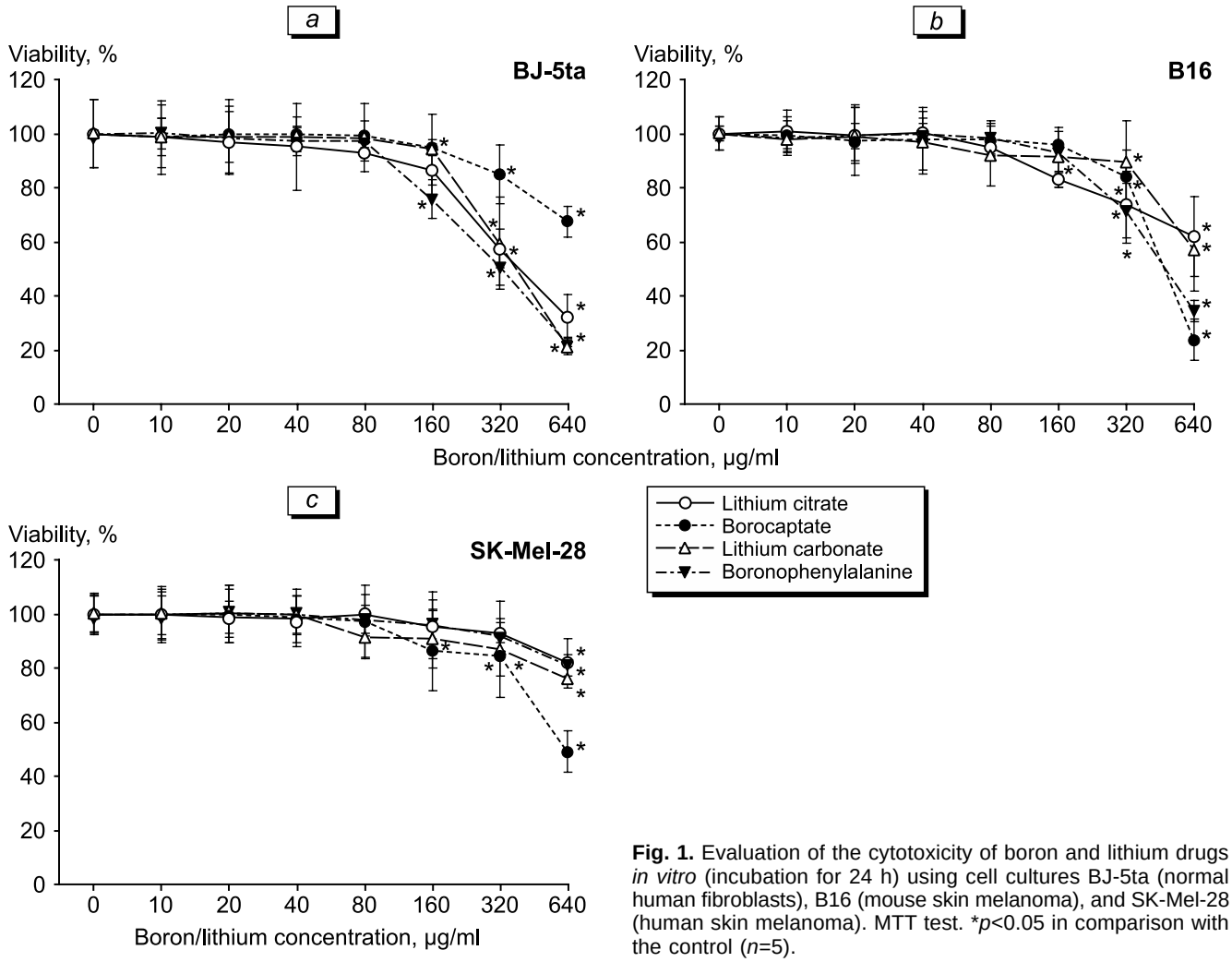

Fig. 1. Evaluation of the cytotoxicity of boron and lithium drugs *in vitro* (incubation for 24 h) using cell cultures BJ-5ta (normal human fibroblasts), B16 (mouse skin melanoma), and SK-Mel-28 (human skin melanoma). MTT test. *p<0.05 in comparison with the control (n=5).

it was shown that boron concentrations in glioma cells 24 h after the administration of boronophenylalanine varied in a range of 0.8-1 μg/10^7 cells [12]; after incubation of 3 melanoma cell lines with boronophenylalanine (boron concentration of 50 μg/ml), boron uptake ranged from 0.04 to 0.12 μg/10^6 cells [13]. The boronophenylalanine administration (30 μg/ml) showed a concentration of 0.2 μg/10^6 T98G human glioblastoma cells [14]; when tumor cells were incubated with boronophenylalanine (50 μg/ml), the concentrations were 1.7 μg/10^7 cells for U251 (human glioma) and 3 μg/10^7 cells for SK-Mel-28 (human melanoma) [15]. Similar results were obtained when glioma cells were incubated with 1 mM borocaptate (0.86 μg/10^7 cells) [16].

It was previously reported that Li^+ enters the cell through Na^+ dependent channels of the plasma membrane [17,18]. When C6 mouse glioma was incubated with lithium chloride for 60 min, the intracellular concentration of lithium was 8 nmol/10^6 cells; it was noted that accumulation of lithium by cells gradually increased over the next few days, which corresponded to the time of clinical response (10-14 days) [10]. When conducting a comparative assessment of lithium accumulation in human neuroblastoma and human glioma cells, significantly higher concentrations of lithium in glioma cells were revealed (up to 90 nmol/mg protein after 40-60 min of incubation with lithium chloride) [11].

In addition, we revealed heterogeneous accumulation of boron and lithium *in vitro* when using boron and lithium drugs. It was previously shown that boron accumulation is variable and depends on the cell line, even when all lines belong to the same histological tumor type [13]. Intratumoral heterogeneity of melanoma cells is known to influence boron uptake and accumulation [19], and tumor heterogeneity can also determine heterogeneous accumulation of lithium in melanoma cells in our study.

In this work, we used lithium salts as non-selective agents to deliver high doses of lithium into tumor cells. The highest lithium uptake values were obtained when B16 murine melanoma cells was incubated with lithium carbonate (0.79 μg/10^6 cells); however,

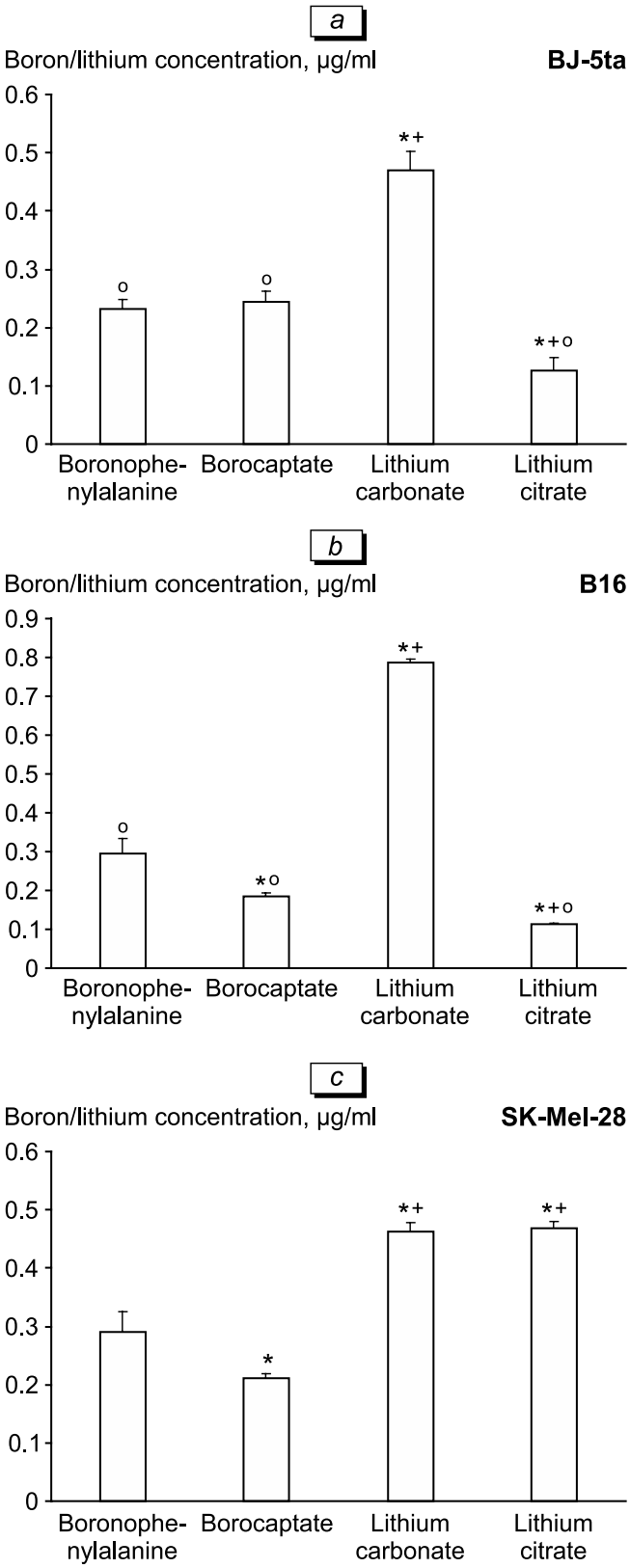

Fig. 2. Concentrations of boron and lithium in cells BJ-5ta (normal human fibroblasts), B16 (mouse skin melanoma), and SK-Mel-28 (human skin melanoma) after 24-h incubation with boron and lithium drugs. $p<0.05$ in comparison with *boronophenylalanine, +borocaptate, °lithium carbonate.

SK-Mel-28 cells also actively accumulated both lithium carbonate and lithium citrate (about 0.46 μg/10^6 cells for the two types of lithium salts). Human fibroblasts absorbed lithium carbonate quite well, while accumulation of lithium citrate was significantly lower. Thus, our results demonstrate a significantly higher uptake of lithium by tumor cells, compared to boron, when incubated with boron drugs approved for clinical use, therefore lithium salts can be used for neutron capture therapy.

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Conflicts of interest. The authors have no conflicts of interest to declare.

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