Radiobiological response of U251MG, CHO-K1 and V79 cell lines to accelerator-based boron neutron capture therapy

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ABSTRACT

In the current article, we provide in vitro efficacy evaluation of a unique accelerator-based neutron source, constructed at the Budker Institute of Nuclear Physics (Novosibirsk, Russian Federation), for boron neutron capture therapy (BNCT), which is particularly effective in the case of invasive cancers. U251MG, CHO-K1 and V79 cells were incubated and irradiated in various concentrations of boric acid with epithermal neutrons for 2–3 h in a plexiglass phantom, using 2.0 MeV proton energy and 1.5–3.0 mA proton current, resulting in a neutron fluence of 2.16 × 10¹² cm⁻². The survival curves of cells loaded with boron were normalized to those irradiated without boron (to exclude the influence of the fast neutron and gamma dose components) and fit to the linear–quadratic (LQ) model. Colony formation assays showed the following cell survival rates (means ± SDs): CHO-K1: 0.348 ± 0.069 (10 ppm), 0.058 ± 0.017 (20 ppm), 0.018 ± 0.005 (40 ppm); V79: 0.476 ± 0.160 (10 ppm), 0.346 ± 0.053 (20 ppm), 0.078 ± 0.015 (40 ppm); and U251MG: 0.311 ± 0.061 (10 ppm), 0.131 ± 0.022 (20 ppm), 0.020 ± 0.010 (40 ppm). The difference between treated cells and controls was significant in all cases (P < 0.01) and confirmed that the neutron source and irradiation regimen were sufficient for control over cell colony formation. We believe our study will serve as a model for ongoing in vitro experiments on neutron capture therapy to advance in this area for further development of accelerator-based BNCT into the clinical phase.

Keywords: boron neutron capture therapy; accelerator-based neutron source; lithium target; boric acid; in vitro efficacy evaluation

INTRODUCTION

Boron neutron capture therapy (BNCT) is a unique radiotherapy method based on the interaction of a stable ¹⁰B isotope with thermal neutrons. The boron neutron capture reaction (¹⁰B(n,α)⁷Li) results in the release of high linear energy transfer (high-LET) alpha and ⁷Li particles, which destroy tumor cell DNA. Selective accumulation of ¹⁰B in cancer cells provides for specific elimination, while sparing normal tissues [1], as the penetration of alpha-particles and
Li nuclei does not exceed single tumor cell depths. Thus, BNCT, in its ideal application, can provide curative treatment for invasive cancers, such as glioblastoma. The efficacy of BNCT from a nuclear reactor neutron source has been confirmed for certain malignancies, including glioma [2–4], malignant melanoma [5], and head and neck cancer [6–9]. However, safety issues, as well as the negative publicity surrounding the Fukushima accident, turned the world BNCT community towards development of accelerator-based neutron sources to replace nuclear reactors in both trials and therapy.

Recently, several accelerators destined for hospital placement have been introduced [10]. For BNCT purposes, a proton accelerator with vacuum insulation and a lithium target have been developed at the Budker Institute of Nuclear Physics (BINP) at the Russian Academy of Sciences (Novosibirsk, Russian Federation) [11]. To the best of our knowledge, no similar accelerator specifically designed for BNCT has ever been created with such unique features. In vitro experiments using tumor and normal cells are typically carried out at the initial biological efficacy evaluation stage. It is within this stage that the main contrast to standard radiotherapy is seen, because BNCT efficacy depends not only on the irradiation source, but also on the accumulation of a boron-containing agent, whose concentration in tumor cells directly influences the treatment effect. In previous experiments at the nuclear reactor, boric acid was used as a standard boron compound for reliable intracellular boron concentration [1].

Any proposed replacement for traditional reactor-based therapies requires pioneering in vitro studies to establish optimum dosages and cellular effects. Therefore, in the current study, we evaluated the efficacy of our accelerator-based neutron source using U251MG, CHO-K1 and V79 cells incubated and irradiated in a boric acid–containing medium at various boron concentrations (0, 10, 20 and 40 ppm), with absorbed dose calculations and further cell survival evaluation using a colony-formation assay (CF assay). Such a method was intentionally used to assure continuous maintenance of boron concentrations during the entire irradiation period, which is one of the key points of these experiments (compared with reports [27–29] for other compounds, where boron was not evenly distributed in the medium and the cells). This study is one of the initial steps of a project on synthesis and evaluation of complex boron/high-Z element compounds for absorbed dose estimation and tumor localization during accelerator-based BNCT.

MATERIALS AND METHODS

Cell lines

Human glioma (U251MG) cells, Chinese hamster ovary cells (CHO-K1), and Chinese hamster lung fibroblasts (V79) were purchased from the Institute of Cytology of the Russian Academy of Sciences (St Petersburg, Russian Federation), cultured in Iscove’s modified Dulbecco’s medium (IMDM) (SIGMA 17633 with L-glutamine and 25 mM HEPES, without sodium bicarbonate), supplemented with 10% fetal bovine serum (Thermo Scientific HyClone SV30160.03 HyClone UK Ltd) and maintained at 37°C in an atmosphere of 5% CO2.

Boric acid application

In vitro experiments were performed at the Institute of Molecular and Cell Biology (Novosibirsk, Russian Federation). The cells were incubated for 2 h in a medium containing boric acid (Sigma-Aldrich, Inc., St Luis, MO, USA) in various concentrations (10, 20 and 40 ppm) of 10B. The cells without boron were irradiated and used as controls. At the indicated time points, medium with boric acid was removed separately for each sample, the cells were washed with phosphate-buffered saline (PBS), trypsinized (0.05% trypsin-EDTA, Nacalai Tesque, Inc., Kyoto, Japan), counted and placed in 2 ml plastic vials in the boric acid–containing medium they were incubated in with the corresponding 10B concentrations (Fig. 1A).

Neutron irradiation

The samples were placed in a phantom made of organic glass at a depth of 3 cm [12, 13] (Fig. 1B) and irradiated in a tandem accelerator with vacuum insulation (Fig. 2A), with the epithermal neutron

Fig. 1. The samples in 2 ml vials (A) placed in the plexiglass phantom (B).
beam under the lithium target (Fig. 2B). The irradiation lasted 2–3 h with the following accelerator settings: 2.0 MeV proton energy, 1.5–3.0 mA proton current (providing an epithermal neutron flux up to $3 \times 10^8 \text{cm}^{-2} \text{s}^{-1}$). The settings were adjusted to produce epithermal neutrons eligible for phantom penetration with subsequent energy decrease to maximize neutron capture by boron in the samples. The necessary depth of the plexiglass in the phantom between the target and the cells was estimated using the Monte Carlo method and provided the maximum thermal neutron irradiation of the samples. The neutron flux was measured by a detector with a lithium-containing scintillator (GS20, Saint-Gobain Crystals, Hiram, OH, USA). Neutron fluence was measured by activation of the gold foil.

Colony-formation assay
After the irradiation, the cells were immediately counted, diluted and seeded into 6 cm dishes for CF assay. After 1–2 weeks, the dishes were washed with PBS, fixed with glutaraldehyde, stained with crystal violet, and dried. Colonies of 50 cells or more were counted for each sample. Cell survival fractions were calculated according to a previously adapted protocol [14, 15]. The results were normalized to controls, which were irradiated without boric acid to smooth the influence of concomitant fast neutrons and gamma-rays, and the statistical significance was evaluated using one-way analysis of variance (ANOVA).

Radiobiological parameters evaluation
The cell survival data were fit to the linear–quadratic (LQ) model, using the SOLVER add-on in Microsoft Excel. As the issue of the absorbed dose evaluation in the accelerator-based BNCT remains controversial, we used the boron concentration instead of the dose to calculate the radiobiological parameters. Using $\alpha'$ and $\beta'$ values, the boron concentration needed to control 90% of cell growth, $C_{10}$ (instead of $D_{10}$), was calculated by solving the following quadratic equation:

$$\alpha' C + \beta' C^2 + \ln(SF) = 0,$$

where $C$ represented $^{10}$B concentration in cells, and in cases with linear survival curves (where $\beta' = 0$) equaled $\frac{\ln(SF)/\alpha'}{2\beta'}$; positive values of $C$ were used.

RESULTS
Colony-formation assay
Three types of cells were incubated in boric acid in four different boron concentrations, which resulted in the analysis of five samples for each cell line in each experiment. The dosages chosen were evaluated as the most likely, realistic concentrations that could be reached in a therapeutic situation. Dishes with stained colonies of each cell line after irradiation with boric acid in the various concentrations are shown in Fig. 3. The number of colonies in all cell lines decreased with increase in boron concentration, with the maximum effect at 40 ppm. Cell survival rates (means ± SDs) are presented in Table 1, and the survival curves are plotted in Fig. 4. The difference between the treated cells and the controls was significant in all cases ($P < 0.01$). This data shows the dosage-dependent effect of boron on the neutron beam and that physiologically relevant concentrations can produce a therapeutic effect.

Radiobiological parameters
The calculated parameters are summarized in Table 2. In two of three cell lines (CHO-K1 and U251MG) $\beta' = 0$, reflecting a linear decrease in cell survival (typical with high-LET irradiation). In the V79 cell line, both $\alpha'$ and $\beta'$ parameters were present, showing different responses of the cell line to similar irradiation regimens. $C_{10}$ values reflected the sensitivity of the cells loaded with boron to...
The reliability of boric acid has been proven in a previously reported radiobiological dosimetry study at the reactor-based neutron source [1]. We assumed a steady state for intra- and extracellular boron concentration, as previously reported data has shown boron distribution throughout bodily fluids in animals and humans to occur in all tissues (except bone) at a concentration not significantly different from that in blood [33–36]. Moreover, we maintained the cellular concentration of boron acid during irradiation by keeping the cells in the medium they were incubated in.

To more accurately model parameters for future studies, we compared the response of several cell lines, including U251MG—a human glioma, and two normal cell lines. First, we chose the CHO-K1 line previously used in our recent study on the accumulation of new boron compounds, and then also examined the V79 cell line (lung fibroblasts that represent normal tissue cells and are generally accepted in the literature as suitable models [1, 28, 32]).

Our cell survival data confirmed the efficacy of the accelerator neutron source with the lithium target at BINP to produce a sufficient number of neutrons to initiate a boron neutron capture reaction within and in proximity to tumor cells. The cell survival rate in each cell line was inversely proportional to the boric acid concentration and observed the nature of the cell response to the irradiation conditions. The cell survival curves were normalized to the data for the cells irradiated without boron to remove fast experimental results of neutron production using a 2 MeV-proton tandem accelerator with vacuum insulation were shown in 2008 [22]. Bayanov et al. have reported on neutron generation experiments on a new 2 MeV tandem accelerator using a specially designed lithium target [23, 24]. It was only then that radiobiological experiments became available (after stabilization of the proton current to 1.5–2 mA was achieved [25] and the size of the accelerator was reduced to make it more compact [26]). It is within this framework of rapid development that we conducted our initial studies to investigate the efficacy of accelerator-based neutron sources.

To date, initial radiobiological experiments on tumor cells to evaluate the efficacy of the neutron source at BINP have been performed with L-p-boronophenylalanine (BPA) [27–29], a boron agent. This compound was previously used as a 10B compound in clinical trials in reactor-based BNCT, along with disodium mercaptopoundecahydrododecaborate (BSH) [30, 31]. However, the results of such in vitro experiments highlighted a critical point; namely, that cellular boron accumulation depends on transport mechanisms and can be influenced by a number of factors, creating the necessity to search for ways to optimize cellular boron levels at those needed for therapy [32]. Such variations in boron compound accumulation can significantly alter the results of a treatment. Further trials might be needed to establish baseline values and reliably predict clinical outcomes.

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We calculated the radiobiological parameters based on the boron concentration and observed the nature of the cell response to the irradiation conditions. The cell survival curves were normalized to the data for the cells irradiated without boron to remove fast

**DISCUSSION**

Recently, a surge in medical interest has seen the development of accelerator-based neutron sources all over the world to replace reactors (for safety and accessibility reasons) [10]. To this end, several types of neutron-producing targets have been introduced: solid 7Li(p,n)7Be after application of a proton beam of 2–2.5 MeV and 10 mA on a lithium target [20]. Further development of the lithium target and the results of the neutron spectrum analysis were reported [21]. The first

**Fig. 3. CF-assay results: 6-cm dishes with stained colonies of each cell line 1–2 weeks after neutron irradiation. CHO-K1 (upper), V79 (middle) and U251MG (lower) cells. The boron concentration is 0, 10, 20 and 40 ppm (from left to right).**

**Table 1. Surviving fractions of irradiated cells**

| Boron concentration (ppm) | 10    | 20    | 40    |
|---------------------------|-------|-------|-------|
| SF V79                    | 0.476 ± 0.160 | 0.346 ± 0.053 | 0.078 ± 0.015 |
| U251MG                    | 0.311 ± 0.061 | 0.131 ± 0.022 | 0.020 ± 0.010 |
| CHO-K1                    | 0.348 ± 0.069 | 0.058 ± 0.017 | 0.018 ± 0.005 |

Cell survival fractions (SFs) are presented as means ± SDs. All SFs significantly differed from controls (P < 0.01, ANOVA).
The differential response between the cell lines could be due to different radiosensitivity or differences in boron uptake. Generally, it might be difficult to exclude the influence of every possible factor, as, regardless of our assumption, some percentage of neutrons may keep higher energies while penetrating the samples, and temperature-dependent neutron scattering cannot be perfectly predicted. Thus, with respect to these unpredictable factors, our methodology to establish correct radiobiological parameters could be counted as limitations of this study.

Longer irradiation times could also affect the overall results due to additional influence from background dose. Therefore, in our experiments, we normalized the responses of cells irradiated with boron to control cells irradiated without boron to specifically exclude the background component. The novelty of our report required us to go above and beyond what would be normal irradiation conditions to discover extreme parameters for both future research and therapy. As technological developments merge with data from multiple analyses, an almost guaranteed shorter irradiation time will be the result as the neutron current increases. This will minimize the background dose effect, and such long exposures will not be typical of future studies.

In our study, we mainly focused on comparison between the effect of the new source and previous data for clinically proven, reactor-based experiments [2]. Therefore, at this stage of development, a baseline comparison was focused on to the exclusion of the cellular mechanisms of response to BNCT or variations in boron uptake (which remain goals for future work). We showed that the accelerator was effective in initial cell experiments and that its performance as a neutron source was close to that of the reactor. For future studies, it will be critical to study detailed parameters of both mechanisms related to boron uptake in different cell lines/animal models and cellular radiosensitivity.

In our experiments, the irradiation effect was obvious for the cells, though the provided epithermal neutron fluence still might be insufficient for clinical trials. In this regard, further improvement of the accelerator, including stabilization of an increased (up to 5 mA) proton current and development of a new lithium target and neutron beam shaping assembly [13] is in progress.

In our study, as in many others, in vitro experiments play only an initial role in evaluation of the method’s efficacy. Animal experiments with appropriate models more adequately and closely represent clinical conditions and will be the main focus of our next set of experiments.

**CONCLUSION**

We carried out an initial evaluation of an accelerator-based neutron source for BNCT at BINP, using boric acid to create verified intracellular boron concentrations that avoided compound accumulation variations. Such variation depends on boron uptake mechanisms and might differ in each cell line. We also proved the ability of the irradiation source to provide sufficient control over cell proliferation after boron uptake. We believe that our study will bring more clarity to ongoing in vitro experiments on neutron capture therapy and help other researchers to advance accelerator-based BNCT into the clinical phase.

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**Fig. 4.** Cell survival curves depend on the boron concentration in the samples. The data are presented as means ± SDs. *P < 0.01 with respect to boron concentration of 0 ppm (one-way ANOVA).

**Table 2. Radiobiological parameters of irradiated cells**

| Cell line | α'   | β'  | C_{10} (ppm) |
|-----------|------|-----|--------------|
| V79       | 0.048| 0.00390192 | 36.90        |
| U251MG    | 0.103| 0    | 22.39        |
| CHO-K1    | 0.123| 0    | 18.69        |

Parameters, such as α' and β' are presented as absolute numbers. C_{10} (in ppm) represents the 10B concentration needed to eliminate 90% of tumor cells.
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CONFLICT OF INTEREST

The authors report no conflicts of interest.

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REFERENCES

1. Yamamoto T, Matsumura A, Yamamoto K et al. Characterization of neutron beams for boron neutron capture therapy: in-air radiobiological dosimetry. Radiat Res 2003;160:70–6.
2. Yamamoto T, Nakai K, Kageji T et al. Boron neutron capture therapy for newly diagnosed glioblastoma. Radiother Oncol 2009;91:80–4.
3. Miyatake S, Kawabata S, Yokoyama K et al. Survival benefit of boron neutron capture therapy for recurrent malignant gliomas. J Neurooncol 2009;91:199–206.
4. Aiyama H, Nakai K, Yamamoto T et al. A clinical trial protocol for second line treatment of malignant brain tumors with BNCT at University of Tsukuba. Appl Radiat Isot 2011;69:1819–22.
5. Yong Z, Song Z, Zhou Y et al. Boron neutron capture therapy for malignant melanoma: first clinical case report in China. Chin J Cancer Res 2016;28:634–40.
6. Kato I, Fujita Y, Maruhashi A et al. Effectiveness of boron neutron capture therapy for recurrent head and neck malignancies. Appl Radiat Isot 2004;61:1069–73.
7. Kankaanranta L, Seppälä T, Koivunoro H et al. Boron neutron capture therapy in the treatment of locally recurred head and neck cancer. Int J Radiat Oncol Biol Phys 2007;69:475–82.
8. Kankaanranta L, Seppälä T, Koivunoro H et al. Boron neutron capture therapy in the treatment of locally recurred head-and-neck cancer: final analysis of a phase I/II trial. Int J Radiat Oncol Biol Phys 2012;82:e67–75.
9. Suzuki M, Kato I, Aihara T et al. Boron neutron capture therapy outcomes for advanced or recurrent head and neck cancer. J Radiat Res 2014;55:146–53.
10. Kreiner AJ, Bergueiro J, Cartelli D et al. Present status of accelerator-based BNCT. Rep Pract Oncol Radiother 2016;21:95–101.
11. Taskaev S. Accelerator based epithermal neutron source. Phys Particles Nucl 2015;46:956–90.
12. Kandiev YA, Kashaeva E, Malyshkin G et al. Optimization of the target of an accelerator-driven neutron source through Monte Carlo numerical simulation of neutron and gamma transport by the PRIZMA code. Appl Radiat Isot 2011;69:1632–4.
13. Zaidi I, Kashaeva E, Lezhnin S et al. Neutron-beam-shaping assembly for boron neutron capture therapy. Phys Atom Nucl 2017;80:60–6.
14. Franken NA, Rodermund HM, Stap J et al. Clonogenic assay of cells in vitro. Nat Protoc 2006;1:2315–9.
15. Zaboronok A, Tsurushima H, Yamamoto T et al. Size-dependent radiosensitization effects of gold nanoparticles on human U251 malignant glioma cells. Nanosci Nanotechnol Lett 2013;5:990–4.
16. Bayanov B, Belov V, Taskaev S. Neutron producing target for accelerator based neutron capture therapy. J Phys Conf Ser 2006;41:460–5.
17. Tanaka H, Sakurai Y, Suzuki M et al. Experimental verification of beam characteristics for cyclotron-based epithermal neutron source (C-BENS). Appl Radiat Isot 2011;69:1642–5.
18. Kreiner AJ, Castell W, Di Paolo H et al. Development of a tandem-electrostatic-quadrupole facility for accelerator-based boron neutron capture therapy. Appl Radiat Isot 2011;69:1672–5.
19. Halfon S, Paul M, Arenshtam A et al. High-power liquid-lithium target prototype for accelerator-based boron neutron capture therapy. Appl Radiat Isot 2011;69:1654–6.
20. Bayanov BF, Belov VP, Bender ED et al. Accelerator based neutron source for the neutron capture and fast neutron therapy at hospital. Nucl Instrum Meth Phys Res A 1998;413:397–426.
21. Bayanov B, Belov V, Kindyuk V et al. Lithium neutron producing target for BINF accelerator-based neutron source. Appl Radiat Isot 2004;61:817–21.
22. Kuznetsov A, Malyskhin G, Makarov A et al. First experiments on neutron registration at accelerator based source for boron neutron capture therapy. Tech Phys Lett 2009;35:1–6.
23. Bayanov B, Kashaeva E, Makarov A et al. A neutron producing target for BINF accelerator-based neutron source. Appl Radiat Isot 2009;67:S282–4.
24. Bayanov B, Burdakov A, Chudaev V et al. First neutron generation in the BINF accelerator based neutron source. Appl Radiat Isot 2009;67:S285–7.
25. Aleynik V, Bashkirtsev A, Kanygin V et al. Current progress and future prospects of the VITA based neutron source. Appl Radiat Isot 2014;88:177–9.
26. Sorokin I, Taskaev S. A new concept of a vacuum insulation tandem accelerator. Appl Radiat Isot 2015;106:101–3.
27. Mostovich LA, Gubanova NV, Kutsenko OS et al. Effect of epithelial neurons on viability of glioblastoma tumor cells in vitro. Bull Exp Biol Med 2011;151:264–7.
28. Volkova OY, Mechetina LV, Taranin AV et al. Impact of neutron radiation on the viability of tumor cells cultured in the presence of boron-10 isotope. Vestn Rentgenol Radiol 2016;97:283–8 (in Russian).
29. Zaboronok A, Byvaltsev VA, Kanygin VV et al. Boron-neutron capture therapy in Russia: preclinical evaluation of efficacy and perspectives of its application in neurooncology. New Armenian Med J 2017;11:1–6.
30. Yamamoto T, Nakai K, Tsurubuchi T et al. Boron neutron capture therapy for newly diagnosed glioblastoma: a pilot study in Tsukuba. Appl Radiat Isot 2009;67:S25–6.
31. Kageji T, Mizobuchi Y, Nagahiro S et al. Clinical results of boron neutron capture therapy (BNCT) for glioblastoma. Appl Radiat Isot 2011;69:1823–5.
32. Sato E, Yamamoto T, Shikano N et al. Intracellular boron accumulation in CHO-K1 cells using amino acid transport control. Appl Radiat Isot 2014;88:99–103.
33. Doras C, Brown PH. Permeability and the mechanism of transport of boric acid across the plasma membrane of Xenopus laevis oocytes. Biol Trace Elem Res 2001;81:127–39.
34. National Center for Biotechnology Information. PubChem Compound Database; CID=7628 Boric Acid. https://pubchem.ncbi.nlm.nih.gov/compound/7628 (2 October 2017, date last accessed).
35. WHO. International Programme on Chemical Safety. Environmental Health Criteria Monographs (EHCs): Boron (ECH 204, 1998). http://www.inchem.org/documents/ehc/ehc/ehc204.htm (2 October 2017, date last accessed).
36. Krieger R (ed). Handbook of Pesticide Toxicology. San Diego, California: Academic Press, 2001, 1413–34.