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ONCOLOGY

Effects of Boron Neutron Capture Therapy on the Growth of Subcutaneous Xenografts of Human Colorectal Adenocarcinoma SW-620 in Immunodeficient Mice

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Boron neutron capture therapy (BNCT) can become an instrument for patients with malignant neoplasms of the rectum and colon. Here we evaluate the effectiveness of BNCT performed at the accelerator based epithermal neutron source at G. I. Budker Institute of Nuclear Physics, Siberian Division of Russian Academy of Sciences, in relation to subcutaneous xenografts of human colon adenocarcinoma SW-620 in SCID mice. Utilization of BNCT with boronophenylalanine (BPA) and sodium borocaptate (BSH), which were injected intravenously into the retroorbital sinus, resulted in a significant decrease in tumor volumes compared to the control group (no radiation).

Key Words: *boron neutron capture therapy; accelerator based epithermal neutron source; boronophenylalanine; sodium borocaptate; human colorectal adenocarcinoma*

Boron neutron capture therapy (BNCT) is considered as one of the promising approaches to treat tumors of colon and rectum [6]. Thus, scientists from Argentina found a significant advantage of the BNCT performed at RA-3 nuclear reactor with boronophenylalanine (BPA) in comparison with γ-radiation on the subline of human colorectal adenocarcinoma [5]. Preclinical tests on BALB/c mice with subcutaneously implanted Colon 26 cell line (mouse colorectal carcinoma) showed tumor regression with its complete disappearance and 100% survival of animals after BNCT at TRIGA-II reactor [10].

In majority of investigations, nuclear reactors were used to generate neutrons, however now active work to create and put into service alternative sources of neutrons is done. One of them is a tandem accelerator with vacuum insulation and a lithium neutron generating target at the G. I. Budker Institute of Nuclear Physics [4]. *In vitro* experiments proved the safety of the neutron beam and its promise for further *in vivo* studies, during which the effectiveness of BNCT was shown against intracranial and subcutaneous tumors

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U87 (human glioblastoma) in immunodeficient animals [1,14].

The aim of this work was to evaluate the effectiveness of boron neutron capture therapy carried out on accelerator based epithermal neutron source at BINP in presence of two boron delivery agents: BPA or sodium borocaptate (BSH) against subcutaneous xenografts of human colon adenocarcinoma SW-620 in SCID mice.

MATERIALS AND METHODS

The study was performed on 18 immunodeficient male SCID (SHO-PrkdcscidHrhr) SPF-status mice at the age of 8-10 weeks, which were kept at the Center for Genetic Resources of Laboratory Animals of the Common Use Center "SPF Vivarium" (Federal Research Center Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences). All animal experiments were carried out with strict adherence to the principles of humane treatment of animals in compliance with the Directive 86/609/EEC (On the Protection of Animals Used for Experimental and Other Scientific Purposes). Culture of SW-620 human colorectal adenocarcinoma cells obtained from the Common Use Center "Center of Genetic Resources of Laboratory Animals" (Federal Research Center Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences) was subcutaneously injected to mice in the area of right thigh (10^6 SW-620 cells in 100 μl suspension) to obtain solid tumors.

Irradiation was performed once 3 weeks after cell implantation, when neoplasms reached volumes of 20-100 μl.

BPA and BSH enriched with ^{10}B isotope (>99.5%, Katchem Ltd.) were used as boron delivery agents. To prepare the BPA solution, fructose (Sigma-Aldrich) was added in molar excess according to the protocol [11]. To obtain a BSH solution, the powder was dissolved in 0.9% NaCl (Renewal). Both drugs were injected 2 h before irradiation intravenously into the retroorbital sinus: BPA in a dose of 350 mg/kg (group 1; n=4), BSH in a dose of 100 mg/kg (group 2; n=4).

Boron concentration in the tumor, blood, kidneys, liver, and brain was measured for further calculation of the "boron dose" as a component of the total dose received by animals. To this end, each preparation was injected intravenously into the retroorbital sinus to mice with subcutaneously transplanted SW-620 tumor (n=3) in the same concentrations as in BNCT animals. In 2 h after injection, the animals were euthanized, the tissues were sampled and processed using optimized methods described earlier [8]; analysis was performed on an iCAP-6500 high-resolution spectrometer (Thermo Fisher Scientific). The mice were intraperitoneally anesthetized with Domitor 0.1% and Zoletil 99.9%. During irradiation, the mice were positioned as described earlier [14]. The animals of the control group were placed in the same conditions as the experimental ones, but without irradiation. Irradiation was carried out for 90 min with the following parameters of the accelerator: proton energy was 2.05 MeV, current integral 3 mAh. To calculate the dose in BNCT, the NMC code was used for the transport of neutral particles in 3D geometry by the Monte-Carlo method [13]. To calculate the equivalent dose, the boron concentration in the organs of interest of the animals was used. The calculated dose in the tumor was 14.7±5.9 Gy-eq for BPA and 6.63±0.53 Gy-eq for BSH. To calculate the dose, we used the CBE (compound biological effectiveness) and RBE (relative biological effectiveness) coefficients taken from [7].

During the observation period, the general well-being, physical activity, and behavior of the animals were assessed every 2-3 days, condition of the skin, both in general and above the tumor, were evaluated. In parallel, the linear dimensions of the tumor were measured and its volume was calculated by the formula [2]: (mean length×mean width^2)×0.52. Inhibition of tumor growth (ITG) was calculated according to the formula [2]: mean volume of the xenograft in the control–mean volume of the xenograft in the experiment)/mean volume of the xenograft in the control×100%. For The tumor growth index (TGI) was calculated as the ratio of xenograft volume at a certain day of the experiment to xenograft volume on day 1 the experiment [2].

For ethical reasons, the mice with tumor volume >4 ml or body weight loss >20% were euthanized. In other cases, euthanasia was performed 60 days after BNCT by CO_2 overdose followed by cervical dislocation.

The data were presented as $M\pm SD$ that were calculated using Microsoft Excel software. The significance of differences between the parameters was determined by the Mann—Whitney U test at $p<0.05$ using the Python 3 programming language (Numpy, Scipy libraries).

RESULTS

Paired comparisons of mean tumor volumes in animals from different groups before irradiation did not reveal significant differences. Analysis of tumor sizes in animals after irradiation showed that tumors progressed most rapidly in the control group (Fig. 1), where a significant difference from the initial values of tumor volumes was observed as soon as on day 9 after BNCT (p=0.016). A significant change in tumor volumes in the BNCT+BSH group relative to the

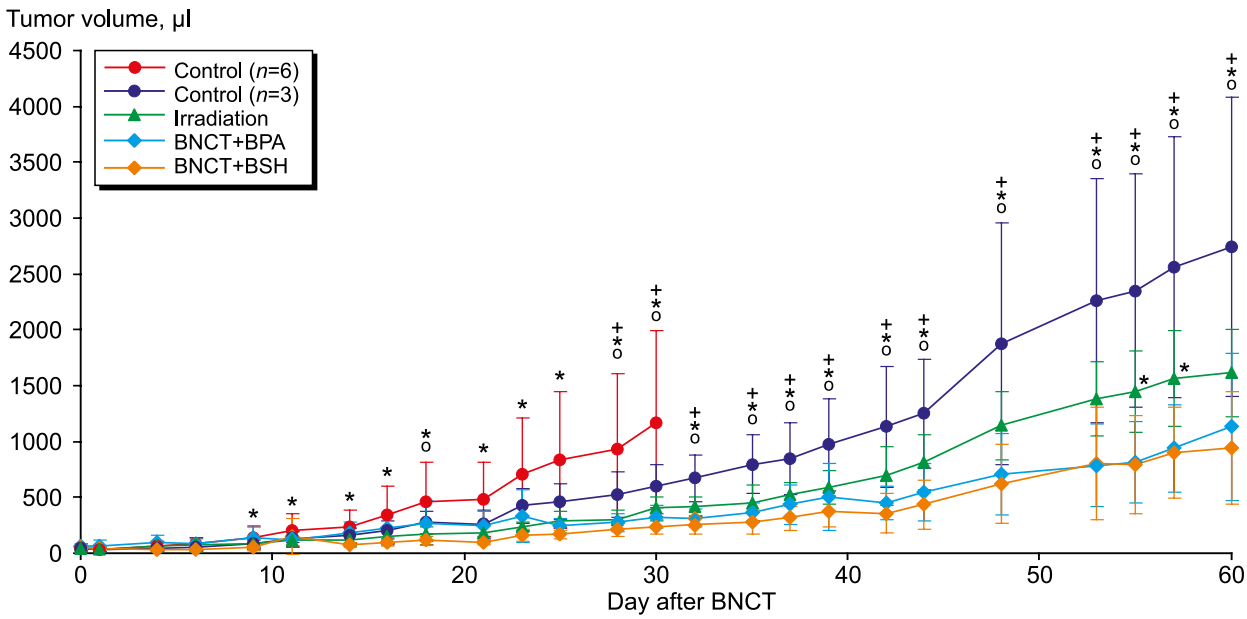

Fig. 1. Dynamics of mean volume of subcutaneous tumors SW-620 in animals of different groups. $p \leq 0.05$ in comparison with *BNCT+BPA group, °BNCT+BSH group, +irradiation group.

initial size was found on day 11 (p=0.043). The slowest tumor growth was observed in the BNCT+BPA group: significant increase was observed only 16 days after BNCT (p=0.043).

Differences in the growth rate of tumors in animals from different groups led to significant differences in their sizes, which were first revealed on day 9 between the control and BNCT+BPA groups (p=0.019), while significant differences between the control and BNCT+BSH groups were observed starting from day 28 (p=0.033) and persisted throughout the entire subsequent observation period (until day 60 after BNCT). The differences between the control group and the irradiation group without boron containing drugs were first revealed on day 18 (p=0.033), but were transient (Figs. 1, 2). Significant differences in the tumor volumes between the BNCT+BPA group and the group with irradiation without boron delivery agents appeared on day 55 after BNCT (p=0.043), persisted up to 57 days (p=0.03), and disappeared on day 60.

To assess the differences between the groups, the entire experiment was divided into 2 periods of 30 days. During the first period, the growth of tumors in the BNCT+BPA group was decelerated by more than 80% (ITG=80.2%); the TG index was 3.2±2.3, which significantly differed from the values obtained in the control group (27.6±18.2; p=0.011) and irradiation group (17.2±7.3; p=0.021). In the BNCT+BSH group, the ITG was 72.4% and the TG index on day 30 from the beginning of experiment was 3.4±0.9 and also significantly differed from the corresponding values in the control group (p=0.011) and irradiation group without boron delivery agents (p=0.021) (Fig. 3).

However, during the second observation period, tumors in the BNCT groups continued to grow: TG index was 21.5±13.9 (p<0.05) in the BNCT+BPA group and 19.5±10.0 (p=0.03) in the BNCT+BSH group (in comparison with the control). The TG index in the control group reached its maximum during the entire observation period: 80.7±49.6, despite the fact that half of the animals in this group were euthanized for ethical reasons on day 30 (Figs. 2, 3). ITG for BNCT+BPA and BNCT+BSH groups in the second observation period was 65.6 and 60%, respectively.

Experiments with laboratory animals can quickly determine the effectiveness of the therapy and improve its quality. However, these studies have some limitations, because the time scale of tumor development in laboratory animals is much shorter than in humans. Previous studies have shown the effectiveness of BNCT performed at reactors against tumors of various localization and histotype [3]. In this work, we analyze the possibility of boron neutron capture therapy for the treatment of colorectal cancer using the beam of epithermal neutrons generated at the accelerator at the G. I. Budker Institute of Nuclear Physics.

For the BNCT+BPA group, the equivalent dose received by the tumor was 2 times higher than in the BNCT+BSH group. This can explain slow growth of the tumors in the BNCT+BPA group during the first month of observation. It should be noted that doses received by other organs and blood (1.7-2.1 Gy-eq) were lower than the equivalent dose received by the

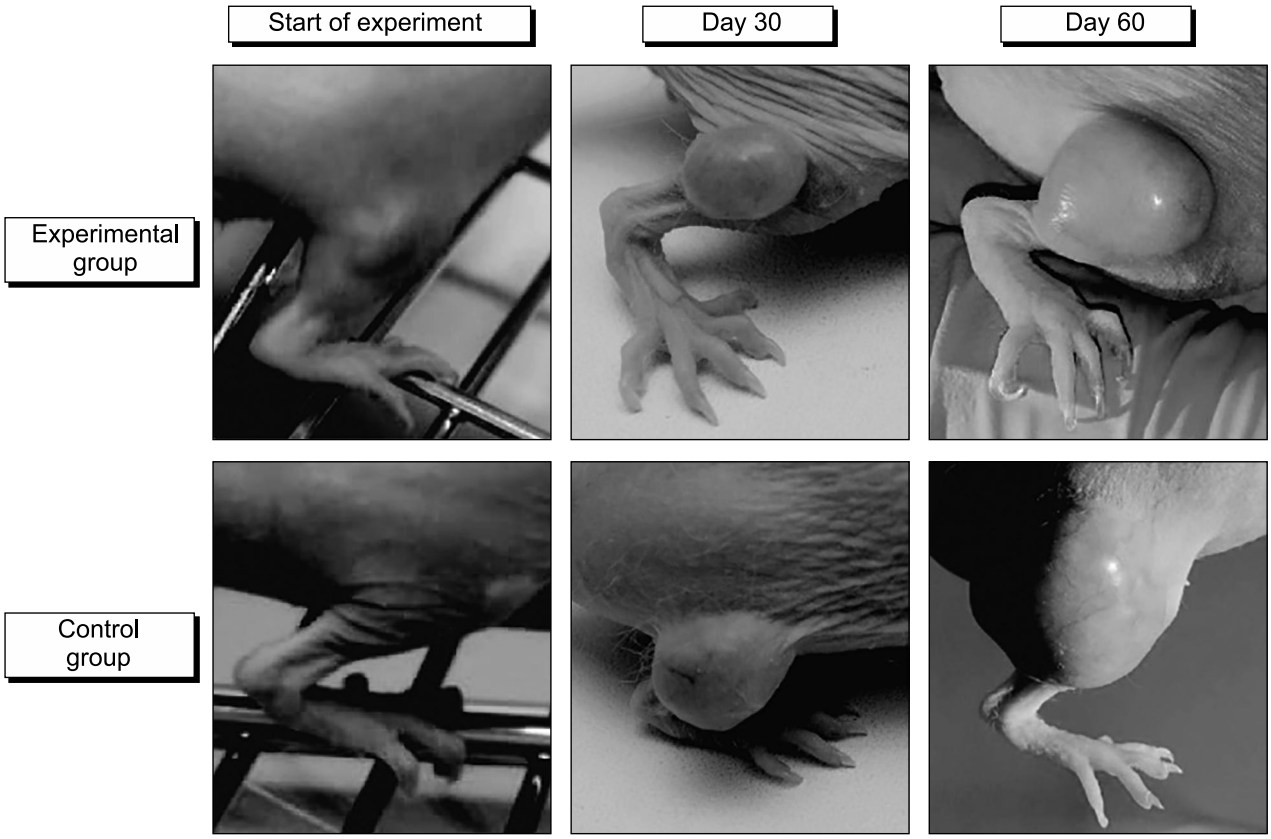

Fig. 2. Tumors in mice from the experimental (BNCT+BPA) and control (without irradiation) groups on the day of BNCT and 30 and 60 days after it.

tumor by 5 times in the BNCT+BPA group and by 3 times in the BNCT+BSH group.

In all experimental groups, the tumors grew much slower than in the control group from day 28 after irradiation. In the irradiated group, the tumors progressed slower than in the control group, but no significant differences were found over the observation period of 30-60 days after therapy, which attested to higher effectiveness of BNCT in comparison with neutron irradiation without boron containing drugs. The maximum tumor size (3000-4000 μl) was achieved in the control group, where the animals were not exposed to radiation. As a result, 50% animals were euthanized for ethical reasons 1 month after the start of the experiment.

The tumor growth in the BNCT+BPA group significantly differed from that in the control group throughout the observation period. However, as noted earlier, the deceleration of tumor growth was more pronounced during the first month after therapy. Probably, the dose received by the tumor was low due to insufficient accumulation of ^{10}B isotope in tumor tissue. This problem can be solved by increasing the concentration of boron containing substances or using an optimal route of its administration. In a previous study conducted on heterotopic U87 xenografts, more

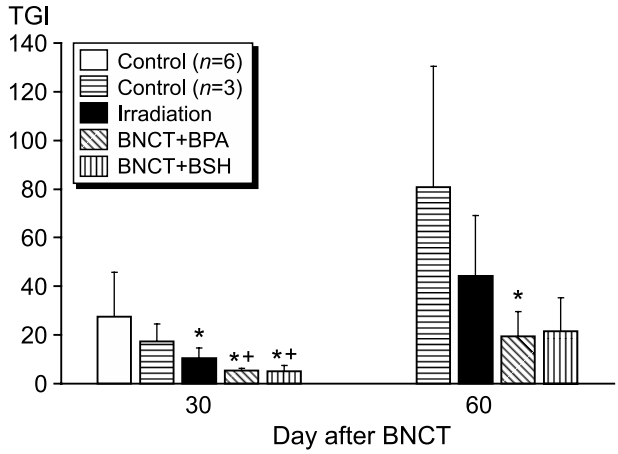

Fig. 3. TGI of subcutaneous SW-620 tumor in animals of different groups. In30 days after the start of the experiment, 50% mice of the control group were euthanized for ethical reasons. $p \leq 0.05$ in comparison with *the control, $^{+}$between BNCT groups and irradiation group (Mann—Whitney *U* test).

intensive accumulation of ^{10}B by the tumor was observed after intratumoral and intraperitoneal administration of boronophenylalanine in comparison with its intravenous administration [8].

Conventional radiation therapy uses radiation dose fractionation to improve tumor growth control

and reduce side effects. In BNCT, the dose is also divided into 2 [9] or 4 fractions [12]. The use of fractionation can be beneficial in further research.

Thus, our *in vivo* study showed that administration of boron delivery agents BPA and BSH followed by irradiation with a flux of epithermal neutrons obtained at the accelerator at the G. I. Budker Institute of Nuclear Physics, Siberian Division of Russian Academy of Sciences, significantly decelerated the growth of human colorectal tumor SW-620 (ITG to 80%), which indicates the prospects of further research in this area of tumor therapy.

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