Marked Low Skin Reaction of Boron Neutron Capture Therapy in Melanoma-Bearing Hamsters in Comparison with a Single-Dose Electron Beam at a Tumor Control Dose

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ABSTRACT. In recent years, boron neutron capture therapy (BNCT) has been established as a special treatment technique for overcoming the radiation resistance of malignant melanomas and brain tumors. There have been several reports on the comparing BNCT and conventional radiotherapy, but very few reports on the differences in normal tissue damage caused by them. The aim of this study was to clarify the differences in skin reactions between BNCT and the electrom beam at a tumor control dose. Hamsters' buttocks transplanted with D178 melanomas were irradiated with the same doses of either thermal neutron beams or 6-MeV electron beams. The skin reactions caused by BNCT were significantly milder than those by electron irradiation, even if the tumor doses were high. These findings lead us to conclude that BNCT is more advantageous than conventional radiotherapy in tumor control without serious damage to the surrounding normal tissue.

Key words : Boron neutron capture therapy (BNCT) — electron beam — melanoma — skin reaction — radiation-resistant tumor

BNCT is based on the fission reaction of $^{10}B(n,\alpha)^{7}Li$, in which ^{10}B atoms incorporated into tumor cells capture thermal neutrons (< 0.5 eV), resulting in the emission of the linear coiling particles α and ^{7}Li (Fig 1). These particles have high linear energy transfer and cause nonrepairable and potentially lethal damage to DNA. In addition, the $^{10}B(n,\alpha)^{7}Li$ reaction in short-range in tissue (~5 μm for ^{7}Li and ~9 μm for the α particle), and surrounding normal tissue theoretically receives hardly any injury.[1-3]

The drug ^{10}B-*p*-boronophenylalanine (BPA) is a boron delivery agent used in clinical trials of BNCT for malignant melanomas (Fig 2).[4] BPA is synthesized as a tyrosine analogue, and is selectively taken up into melanoma cells as a precursor of melanin. However, the radiation injury of the tumor-surrounding normal tissue is not negligible even in BNCT, because normal tissues also take up BPA to some extent.

Many reports have reviewed the tumor-suppressive effects of BNCT on malignant melanomas and brain tumors,[1-8] but there have been few reports comparing damage to surrounding normal tissue under the condition of tumor-absorbed doses which produce the same tumor response in BNCT and

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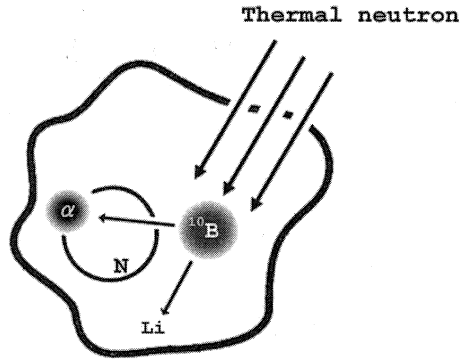

Fig 1. The principle of boron neutron capture therapy (BNCT). α : α particle ; ^{10}B : ^{10}B atom ; Li : ^{7}Li particle ; N : nucleus of the cell.

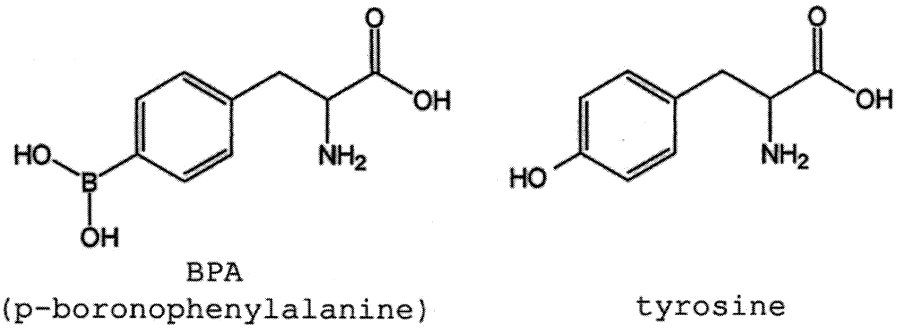

Fig 2. Structures of the boron compound BPA and tyrosine.

conventional irradiation.[9-11] The purpose of this study was to clarify the differences in tumor-surrounding normal skin response at a tumor control dose between BNCT and single-dose electron irradiation.

MATERIALS AND METHODS

Tumor Model

We used hamster D178 amelanotic melanoma (obtained from Dr. H.S.N. Greene [1958][12]), which is considered a human melanoma counterpart from the view points of biology and pathology. D178 amelanotic melanomas (2 mm in diameter) were transplanted subcutaneously into the buttocks of six-week-old female Syrian (golden) hamsters. The experiments were performed on the 14th day after transplantation when the melanomas had grown to 15 to 20 mm in diameter.

^{10}B Compounds

BPA, which was enriched with 95% ^{10}B, was obtained from STELLA CHEMIFA Corp., Osaka, Japan, and was used as a melanoma-seeking ^{10}B compound. Since it is relatively insoluble at physiological pH, Yoshino *et al*[13,14] developed a BPA-fructose complex to make it soluble.

Kinetics of BPA-Uptake in Melanoma and Normal Skin

The melonoma-bearing hamsters received a single intraperitoneal injection of BPA as a BPA-fructose complex (400 mg/kg body weight). Then, the melanomas and surrounding normal skin were resected from the hamsters under anesthesia at 0.5, 1, 2, 3, and 6 h after BPA administration. Each sample was solubilized in a mixture consisting of $HClO_4$ (60%, 0.9 ml) and H_2O_2 (30%, 1.8 ml). After these solubilized samples were filtered by a 0.45-μm filter, the boron concentration in the tumors and normal skins were assayed by inductively coupled plasma-atomic emission spectrometry (ICP-AES) at STELLA CHEMIFA Corp.

Thermal Neutron Irradiation for Melanoma-bearing Hamsters

As a thermal neutron beam source, the Kyoto University Research Reactor (KUR) was used. The thermal column of the KUR has a thermal neutron flux of 5.4×10^{8}n/cm^2/s/MW. The hamsters were intraperitoneally administered BPA as a BPA-fructose complex (400 mg/kg body weight). Two hours after BPA administration, they were held under anesthesia within a specially designed acrylic cage. The body of each hamster, except for the melanoma-bearing region of the buttocks, was shielded from the thermal neutrons by LiF tiles.[9] They were placed 10 cm from the bismuth surface of the reactor. Subsequently, the hamsters were irradiated with thermal neutrons of the KUR at 5 MW for 40 min (n=6).

The thermal neutron fluences and γ-rays on the surface of the tumors were measured by gold-foil activation analysis and a thermo-luminescence dosimeter (TLD) of Mg_2SiO_4, respectively. The total absorbed dose resulting from the thermal neutron beam was the sum of the absorbed doses from the $^{10}B(n,\alpha)^7Li$ reaction, the $^{14}N(n,p)^{14}C$ reaction, the $^{1}H(n,\gamma)^2H$ reaction, and the primary and capture γ-rays. Each of the absorbed doses was calculated from the following equation :[15]

$$D=G+(6.782\times10^{-14}\ N\ R_N+7.436\times10^{-14}\ B\ R_B)\Phi$$

in which D = the absorbed dose in relative biological effectiveness (RBE) − Gy ; G = the absorbed γ-ray dose measured directly by TLD (Gy) ; 6.782×10^{-14} = conversion factor from the $^{14}N(n,p)^{14}C$ reaction to Gy :N = the fraction by weight of nitrogen in the tissue (the concentration was 3.483%[16]) ; R_N = the RBE of the $^{14}N(n,p)^{14}C$ reaction ; 7.436×10^{-14} = the conversion factor from the $^{10}B(n,\alpha)^7Li$ reaction to Gy ; B = the number of ^{10}B atoms per gram of tissue ; R_B = the RBE of the $^{10}B(n,\alpha)^7Li$ reaction ; and Φ = the fluence of neutrons (n/cm^2).

Electron Irradiation for Melanoma-bearing Hamsters

The lesions of melanoma-bearing hamsters were irradiated with 6-MeV electron beams. The dose rate of the electron beams was 2.5 Gy/min, the field size was 20×20 cm and the focus-to-skin distance was 100 cm. The remainder of the body area was shielded by a lead board. They were irradiated for 30 Gy (n=4).

Evaluation of Radiation Effects

The results of the treatment were monitored by measuring the tumor size as a function of time post-irradiation. Tumor volume was calculated

TABLE 1. System for scoring skin reaction

Score	Reaction
0.0	No apparent difference from normal
0.5	Slight reddening
1.0	severe reddening and/or dry desquamation (+)
1.5	Dry desquamation (++)
2.0	Moist desquamation of a small area
2.5	Moist desquamation of a large area
3.0	Moist desquamation of most of the skin of the irradiated area
3.5	Ulceration of the irradiated area

according to Van Woert and Palmer's equation, $V=\Pi/4\ (d_1)\ (d_2)\ (d_3)$.[17]

Skin reactions were recorded twice a week from the 1st to the 30th day after irradiation, according to a modified scale (Table 1) published by Fowler *et al.*[18]

Statistical analysis

For statistical analysis, the Mann-Whitney U test was used. Differences were considered significant if $P<0.05$.

The animal protocol for this experiment was approved by the Animal Care and Use Committee of Kawasaki Medical School (No.02-109, 2002, No.03-083, 2003).

RESULTS

Boron Concentration in Melanoma and Normal Skin

Fig 3 shows the time course of the boron concentration in the melanomas and normal skins. The boron concentrations in the melanomas

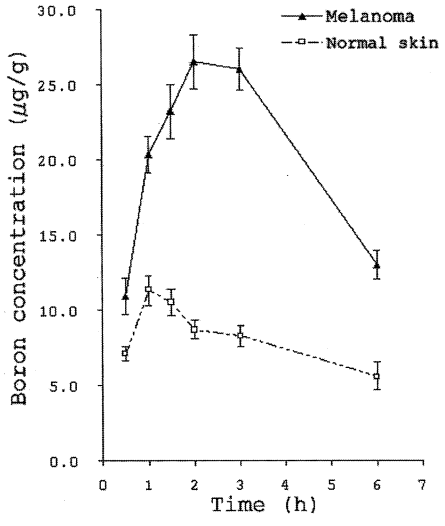

Fig 3. Time course of boron concentration in melanoma and normal skin. Each point represents the mean of five measurements±standard error (SE).

and normal skins at 2 h after BPA administration were 26.5±1.8 μg ^{10}B/g and 8.7±0.6 μg ^{10}B/g, respectively. The tumor/normal tissue (T/N) ratio of ^{10}B concentration was approximately 3.1 at this point. The boron concentration of the tumors reached a peak at 2 h after administration of the BPA. On the other hand, that of normal skin reached a peak at 1 h.

Absorbed Doses of Melanoma and Skin after BNCT

The thermal neutron fluences, determined by gold-foil activation analysis, were 5.7×10^{12} n/cm^2. Assuming that the RBE of the high LET irradiation was 2.5,[9] the average absorbed doses of the melanomas and normal skins calculated from the above data were 32.7 and 13.9 RBE-Gy, respectively (Table 2).

Tumor-Suppressive Effect after Irradiation

The tumor growth curve after thermal neutron irradiation is shown in Fig 4A. The tumor volume rate at each time point for each hamster was based on the initial tumor volume. The growth curves of electron irradiation were plotted in a similar manner (Fig 4B).

TABLE 2. Absorbed dose of neutron capture therapy

region	Absorbed dose (RBE-Gy)			
	^{10}B(n,α)^{7}Li*	^{14}N(n,p)^{14}C*	γ-Ray**	Total
tumor	28.0	3.4	1.3	32.7
skin	9.2	3.4	1.3	13.9

*The RBE of ^{10}B(n,α)^{7}Li and ^{14}N(n,p)^{14}C=2.5.
**Primary and capture γ-rays, and the RBE of γ-rays=1.0.

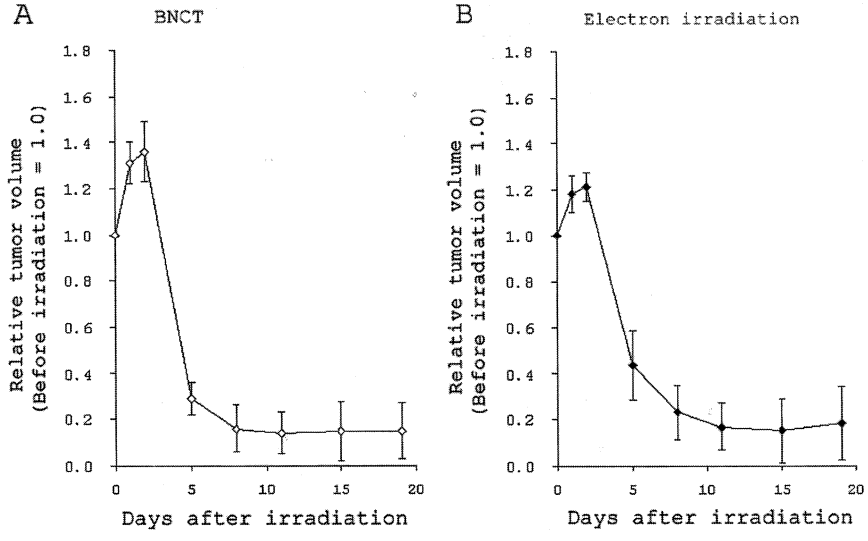

Fig 4. The tumor-suppressive effect after irradiation. A) The growth curves for hamster melanoma irradiated with 6-MeV electron beams, and B) for those irradiated with thermal neutron beams. The symbols rapresent the mean of 4-6 measurements ± standard error (SE).

The BNCT group and electron irradiation group presented similar tumor growth curves after irradiation. The tumor volumes decreased sharply from the 2nd to the 5th day after irradiation and decreased slowly afterwards. Tumor regrowth was not found within the duration of observation.

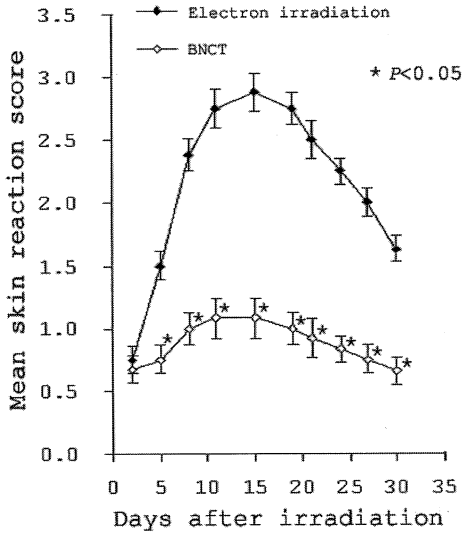

Fig 5. Average skin reactions of hamsters after single irradiation with thermal neutron beams, compared to those with 6-MeV electron beams. Thermal neutron irradiation significantly reduced skin reactions in comparison with electron irradiation after the fifth day ($p<0.05$). The symbols represent the mean of four to six measurements ± standard error (SE).

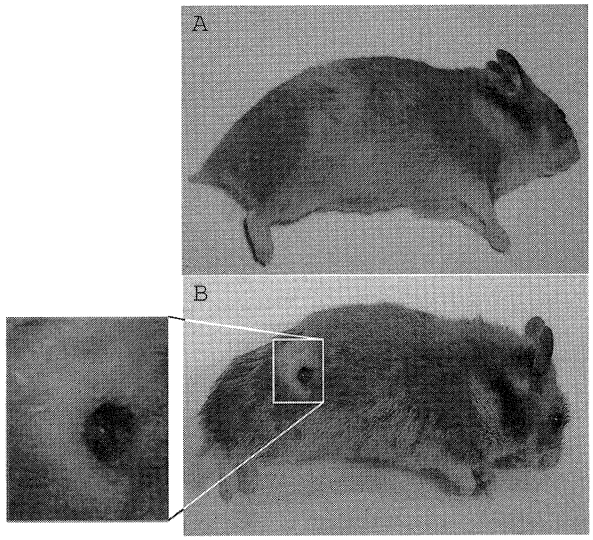

Fig 6. The state of the skin after irradiation. A) A hamster treated with thermal neutron irradiation for 40 min, and B) one treated with electron irradiation of 30 Gy. In the electron irradiation group, a cutaneous ulcer formed in the irradiation locus. In the BNCT group, however, no ulceration of normal skin was found.

Skin Reactions after BNCT Compared with Those after Electron Irradiation

The average skin reaction scores after irradiation are plotted in Fig 5. The skin reactions of the BNCT group were significantly lower than those of the electron irradiation group ($P < 0.05$). Fig 6A and 6B show the marked low skin reaction in the BNCT group on the 19th day after BNCT.

DISCUSSION

For radiotherapy, the clinical safety limit of skin damage is thought to be dry desquamation, and Hiratsuka *et al*[9] reported the single irradiation dose required to produce dry desquamation in hamster skin is 20.6 Gy. In the present study, the actual skin reactions caused by BNCT were milder than those by electron irradiation under the condition of the tumor control doses. In addition, our results obtained with BNCT were within tolerable limits of skin damage. These results lead us to conclude that BNCT is more advantageous than conventional radiotherapy in tumor control without serious damage to the surrounding normal tissue.

The boron content in the tumor cells strongly influences the results of BNCT.[4] Several authors have reported that there is a close correlation between BPA accumulation and melanin-producing activity in melanoma cells.[19-21] However, our results suggest that other factors may affect the BPA accumulation of melanoma cells. The reason for the high accumulation of BPA in amelanotic melanomas is not well known, but it may involve an increase in the metabolic demand for amino acids. In *in vitro* experiments with non-melanoma cells, Witting *et al*[22] proved that BPA was transported into the cells by the L amino acid transport system.

Another important factor that has a strong influence on the results of BNCT is the T/N tissue boron concentration ratio. If the T/N ratio of the boron concentration is higher, the dose absorbed by normal tissue can be suppressed more while keeping the tumor dose high. As shown, in Fig 3, the time to reach the maximum boron concentration was different between the tumors and normal skins. Therefore, the T/N ratio changes with time. This result strongly shows that the time after BPA administration plays an important role in the therapeutic gain of BNCT. The T/N ratio reached its peak at 2 h after administration, and the high value was maintained for up to 3 h. The good tumor control without serious damage to the surrounding normal tissue was obtained by irradiation with thermal neutrons during this period of time. Smith *et al*[23] reported that a 6-h or longer continuous intravenous infusion of BPA raised boron levels in tumor cells and improved the T/N ratio in a rat brain tumor model. Further investigations are required to improve the BPA uptake and T/N ratio of cancer cell lines. We have studies in progress for improving the selectivity of BPA uptake into melanoma cells by transfection of the tyrosinase gene into these cells, to change the characteristics of the cells themselves.

Some institutions have reported that BPA is accumulated in non-pigmented tumors, too, such as thyroid carcinomas, malignant parotid gland tumors and oral squamous-cell carcinomas. Currently, *in vivo* studies and clinical trials of BNCT for these tumors using BPA are being conducted.[24-28] The clinical application of BNCT as a new form of radiation therapy for

such radiation-resistant tumors is expected.

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