Jpn. J. Cancer Res. **89**, 81–88, January 1998

Responses of Total and Quiescent Cell Populations in Solid Tumors to Boron and Gadolinium Neutron Capture Reaction Using Neutrons with Two Different Energy Spectra

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In neutron capture therapy, whose effectiveness depends on the tumor distribution of neutron capture compound and the neutron energy distribution, controlling quiescent tumor cells with clonogenic potential is critical for therapeutic gain, as is the case in conventional radio- and chemotherapy. Tumor-bearing mice were continuously given 5-bromo-2′-deoxyuridine (BrdU) to label all proliferating cells. After administration of sodium borocaptate-^{10}B (BSH), *dl-p*-boronophenylalanine-^{10}B (BPA) or gadodiamide hydrate (Omniscan), the tumors were irradiated with neutrons of different cadmium (Cd) ratio, and then isolated and incubated with cytochalasin-B (a cytokinesis blocker). The micronucleus (MN) frequency in cells without BrdU labeling (quiescent cells) was determined using immunofluorescence staining for BrdU, and that for total cells was obtained from tumors not pretreated with BrdU. Without drugs, quiescent cells showed lower MN frequencies than total cells, but neutron irradiation reduced gamma-ray sensitivity difference between the two. Relative biological effectiveness (RBE) of neutrons compared with gamma-rays was greater in quiescent cells than in total cells, and low Cd ratio neutrons tended to exhibit large RBE values. With neutron capture compounds, MN frequency for each cell population was increased, especially when high Cd ratio neutrons were used. BPA increased the MN frequency for total cells to a greater extent than BSH. However, the sensitivity of quiescent cells treated with BPA was lower than that in BSH-treated quiescent cells. This tendency was clearly observed in high Cd ratio neutrons. Omniscan only slightly increased the MN frequency in both cell populations, compared with irradiation alone, without drugs. From the viewpoint of increasing the quiescent cell sensitivity, tumors should be irradiated with high Cd ratio neutrons after BSH administration.

Key words: Quiescent cell — Micronucleus — Neutron capture reaction — Cadmium ratio

Neutron capture therapy (NCT) has the potential to deliver radiation more selectively than is the case with conventional radiotherapy. Boron NCT is based on the reaction that occurs between the ^{10}B nucleus and thermal neutrons, and represents a promising modality for selective irradiation of tumor tissue. ^{10}B nuclei capture slow thermal neutrons preferentially, and upon capture promptly undergo nuclear fission. The heavy charged particles produced by this $^{10}B(n,\alpha)^{7}Li$ reaction have ranges of ≈9 μm and ≈5 μm, respectively, in tissue and have a high relative biological effectiveness (RBE) for controlling tumor growth as compared with gamma radiation.[1] Gadolinium neutron capture reaction results in the emission of gamma-rays with the maximum energy of 7.9 MeV followed by a series of secondary gamma-rays and 29 to 180 keV internal conversion electrons.[2] On such gadolinium (Gd) NCT, internal conversion electrons and Auger electrons are thought to play important roles in microscopic energy deposition, and these electrons are responsible for about 15% of the total absorbed dose in Gd-treated tumors.[3]

Many tumor cells are not proliferating (quiescent) *in situ*, but are still clonogenic.[4] To improve NCT, it is thus necessary to determine the response of quiescent (Q) tumor cells to this treatment. Until recently, a comparatively simple assay for assessing the response of intratumor Q cells was not available. In order to analyze the responses of Q cells in solid tumors, we have developed a combined method with micronucleus (MN) frequency assay and identification of proliferating (P) cells by 5-bromo-2′-deoxyuridine (BrdU) and anti-BrdU monoclonal antibody.[5] With this method, we analyzed the killing effects of the neutron capture reaction using neutrons with two different energy spectra on Q cells within murine solid SCC VII squamous carcinoma, in terms of the MN frequency, in comparison with those in the total (P+Q) tumor cell populations. The reaction was performed with two ^{10}B-enriched compounds, $Na_2B_{12}H_{11}SH$ (sodium borocaptate-^{10}B, BSH) and *dl-p*-boronophenylalanine-^{10}B (BPA), which have been used clinically in Japan.[1] Non-enriched Gd in the form of Omniscan (gadodiamide hydrate; Daiichi Pharmaceutical Corporation, Tokyo), which has been employed clinically as a nonionized contrast agent for magnetic resonance imaging, was also used.

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MATERIALS AND METHODS

Tumors, mice, and labeling with BrdU SCC VII squa-

mous cell carcinoma, derived from C3H mice, was maintained *in vitro* in Eagle's minimum essential medium containing 12.5% fetal bovine serum. Cells were collected from monolayer cultures, and approximately 1.0×10^5 cells were inoculated subcutaneously into the left hind legs of 8- to 11-week-old syngeneic female C3H/He mice. Fourteen days after inoculation, the tumors had reached approximately 1 cm in diameter. Nine days after inoculation, mini-osmotic pumps (Alzet model 2001, Polo Alto, CA) were implanted subcutaneously for 5 days of continuous labeling. Administration of BrdU did not change the tumor growth rate. The tumors were 1 cm in diameter at treatment. The labeling index after 5 days of continuous labeling with BrdU was 55.3±4.5% (mean±SD), and reached a plateau at this stage. Therefore, in this study, we regarded tumor cells not incorporating BrdU after continuous labeling as Q cells.

Treatment A LiF thermoplastic box holding three mice was made, and BrdU-labeled tumor-bearing legs were pulled out of the box through a narrow slit in the front side. The legs were fixed with adhesives. Twenty minutes after the intraperitoneal injection of BSH (125 mg/kg) dissolved in physiological saline, 3 h after oral administration of BPA (1,500 mg/kg) also dissolved in physiological saline, or 30 min after the intraperitoneal injection of Omniscan (40 ml/kg, 12.92 g/kg), the tumors were irradiated with a neutron beam generated by the Kyoto University Reactor (KUR).[6] We used neutron beams with two different cadmium (Cd) ratios which have become available through remodeling of the heavy water facility at KUR from August, 1995, to May, 1996.[7] Essentially, this remodeling was performed mainly to meet the following requirements: 1) clinical utilization of the heavy water facility during continuous KUR operations, and 2) utilization of epithermal neutron irradiation for the treatment of deep-seated tumors. Cd ratio represents the degree to which the thermal neutron beam is contaminated with fast neutrons, and refers to gold foil activation with and without Cd covers on the gold foils (Cd stops all thermal neutrons). A high Cd ratio means that the beam has a high fraction of thermal neutrons. Cd ratios of the employed neutron beams were 148 and 1.0. The neutron fluence was measured with a phantom by radioactivation of gold foil (3 mm diameter; 0.05 mm thickness) both at the front and back sides of tumors. Gamma-ray doses, including secondary gamma-rays, were measured with thermoluminescence dosimeter powder at the back side of tumors. For estimation of neutron energy spectra, eight kinds of activation foil and fourteen kinds of nuclear reaction were used: $Au^{197}(n,\gamma)Au^{198}$ for the thermal neutron region, $^{115}In(n,\gamma)^{116m}In$, $^{197}Au(n,\gamma)^{198}Au$, $^{58}Fe(n,\gamma)^{59}Fe$ and $^{63}Cu(n,\gamma)^{64}Cu$ for the epithermal neutron region, and $^{115}In(n,n^*)^{115m}In$, $^{54}Fe(n,p)^{54}Mn$, $^{27}Al(n,p)^{27}Mg$, $^{27}Al(n,\alpha)^{24}Na$, $^{47}Ti(n,p)^{47}Sc$, $^{48}Ti(n,p)^{48}Sc$, $^{24}Mg(n,p)^{24}Na$, $^{63}Cu(n,\alpha)^{60}Co$, $^{58}Ni(n,p)^{58}Co$ and $^{197}Au(n,2n)^{196}Au$ for the fast neutron region. Neutron absorbed dose was calculated by using the flux-to-dose conversion factor.[8] Weight percentage of the tumors was assumed to be H (10.7%), C (12.1%), N (2%), O (71.4%) and others (3.8%).[9] Since the tumors were small and located just beneath the surface, intratumor neutron fluence was assumed to be linearly decreased from the front to the back side of the tumors. Thus, we used the averaged neutron fluence from the measured values at the front and back sides of tumors. Averaged neutron fluence and Kerma dose rate, and measured gamma-ray dose rate for each irradiation mode are shown in Table I. Meanwhile, for gamma-ray radiation as a control, we used a cobalt-60 gamma-ray irradiator at a dose rate of 5.97 Gy/min.

On the other hand, each treatment group also included mice that were not pretreated with BrdU.

Some of the tumors that were not irradiated were used to determine ^{10}B and ^{157}Gd concentrations in the tumors. The ^{10}B and ^{157}Gd concentrations in the tumors were measured by prompt gamma-ray spectrometry using a thermal neutron guide tube at KUR.[10]

Immunofluorescence staining of BrdU-labeled cells and observation of micronucleus formation These procedures have been described in detail elsewhere.[5] After the above treatments, excised tumors from mice given BrdU were minced and trypsinized at 37°C for 15 min, using 0.05% trypsin and 0.02% ethylenediaminetetraacetic acid. Tumor cell suspensions were inoculated into 60-mm tissue culture dishes containing 5 ml of complete medium and 1.0 μg/ml of cytochalasin-B to inhibit cytokinesis while allowing nuclear division. The proportion of binuclear cells reached a maximum 48 h and 72 h after initiation of culture for total and Q cell populations, respectively. The cultures were trypsinized and single-cell suspensions were fixed with 70% ethanol. After centrifugation, the cell pellets were resuspended with 0.4 ml of cold Carnoy's fixative. The suspensions (30 μl) were then placed on glass microscope slides using a dropper and dried at room temperature. The slides were treated with 2 *N* hydrochloric acid for 30 min at room temperature to dissociate the histones and to partially to denature the DNA. The slides were then immersed in borax-borate buffer (pH 8.5) to neutralize the

Table I. Neutron Fluence (n/cm^2s) and Kerma Dose Rate (cGy/h) for Each Neutron Irradiation Mode

Cadmium ratio	Thermal neutrons (–0.6 eV)	Epithermal neutrons (0.6 eV–10 keV)	Fast neutrons (10 keV–)	Gamma-rays dose rate (cGy/h)
148	2.0×10^9	2.8×10^7	6.6×10^6	
	96	1.03	28.4	120
1.0	—	8.0×10^8	4.7×10^7	
	—	23	160	70

acid. BrdU-labeled cells were detected by indirect immunofluorescence staining using monoclonal anti-BrdU antibody and fluorescein isothiocyanate (FITC)-conjugated antimouse IgG. To observe the double-staining of tumor cells with FITC and propidium iodide (PI), cells on the slides were treated with 30 μl of PI (1–5 μg/ml in phosphate-buffered saline) under a fluorescence microscope. When the intensity of the red fluorescence produced by PI became similar to the intensity of the green fluorescence in nuclei prestained with FITC, the treatment was stopped by rinsing the slides with water. The MN frequency in BrdU-unlabeled cells (= Q cells) could be examined by counting the micronuclei in those binuclear cells that showed only red fluorescence. The MN frequency was defined as the ratio of the number of micronuclei in the binuclear cells to the total number of binuclear cells observed.[11] The ratio obtained in tumors not pretreated with BrdU indicated the MN frequency of all phases of the total tumor (P+Q) cell populations.

The MN frequency of BrdU-labeled cells, which could be regarded as P cells upon treatment, was modified because the radiosensitization effect of the incorporated BrdU[12] has the potential to influence the frequency of micronucleus and binuclear cell appearance in BrdU-labeled cells. Therefore, the correct MN frequency of P cells without the BrdU effect could not be obtained. In addition, during continuous labeling with the BrdU over 5 days, the shift of cells from P to Q population resulted in labeled Q cells. These cells were excluded when we scored micronuclei in binuclear cells showing only red fluorescence by PI, because these cells were stained with FITC.

Four mice were used for each set of conditions and each experiment was repeated 3 times. To examine the differences between pairs of values, Student's *t* test was used when variances of the two groups could be assumed to be equal; otherwise, the Welch *t* test was used.

RESULTS

The ^{10}B concentrations in tumors in the BPA and BSH administration groups were 10.7±2.8 μg/g (1.1±0.3 m*M*) and 11.2±1.2 μg/g (1.1±0.1 m*M*), respectively, and this difference was not significant. ^{157}Gd concentration in tumors for Omniscan administration was 468.5±52.8 μg/g (3.0±0.3 m*M*). Table II shows the MN frequencies without radiation for total tumor cells and for Q cells. With or without neutron capture compounds, the MN frequency of Q cells was higher than that of total cells when no radiation was delivered.

Figs. 1 and 2 show the normalized MN frequencies for each irradiation condition as a function of the absorbed radiation dose in total tumor cells and in Q cells, respectively. When a neutron capture compound was administered before tumor excision, even if no radiation was given, the MN frequency was higher than when no drug was administered, because of the genotoxicity of the drug (Table II). Therefore, for baseline correction, we used the normalized MN frequency to exclude the effects of the genotoxicity of the neutron capture compound on the MN frequency. The normalized MN frequency is the MN frequency in the irradiated tumors minus the MN frequency in the nonirradiated tumors.

Without neutron capture compounds, the normalized MN frequency in Q cells was lower than that in the total cell population, especially in the case of gamma-ray irradiation. We calculated the dose-modifying factors (DMFs) of Q cells in tumors not treated with a neutron capture compound; these factors were used to compare the radiation doses necessary to obtain various normalized MN frequencies in Q cells with the doses required in the total tumor cell population. For this calculation, we used the values from tumors excised after irradiation alone without neutron capture compound administration, as shown in Figs. 1 and 2 (Table III). The values of DMF for gamma-rays were greater than 2.00, and significantly higher than 1.00 ($P<0.05$). In contrast, with neutrons of each Cd ratio, the values were closer to 1.00 than those for gamma-rays. To examine the RBE of irradiation with neutrons, DMFs, which compare the radiation doses necessary to obtain various normalized MN frequencies in tumors irradiated with gamma-rays with those in tumors irradiated with neutrons without neutron capture compounds in each cell type, were calculated using the mean values of the data shown in Figs. 1 and 2 (Table IV). These values of DMFs were equivalent to those of RBE. All were significantly greater than 1.00 ($P<0.05$), and the values for Q cells were significantly larger

Table II. Micronucleus Frequency at 0 Gy

Cell fraction	No drug	BSH[a]	BPA[b]	Omniscan[c]
Total tumor cells	0.025±0.003	0.034±0.004	0.048±0.009	0.031±0.004
Quiescent tumor cells	0.053±0.004	0.071±0.010	0.068±0.010	0.060±0.008

a) Sodium borocaptate-^{10}B.
b) *dl*-*p*-Boronophenylalanine-^{10}B.
c) Gadodiamide hydrate.

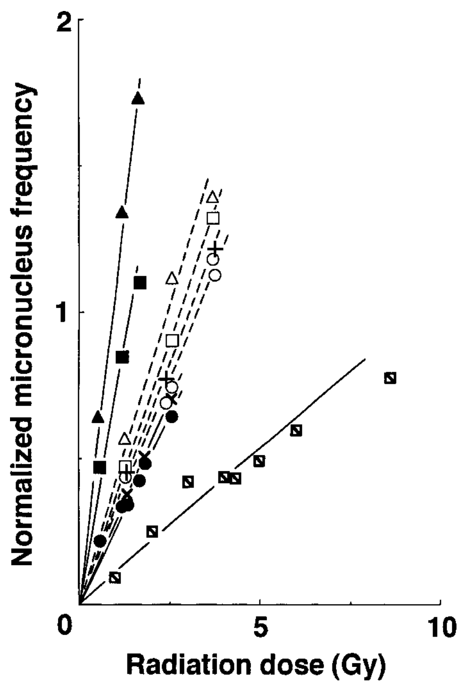

Fig. 1. Dose-response curves of normalized MN frequency as a function of radiation dose for total tumor cell populations. Tumors were irradiated using neutrons with a Cd ratio of 148 (●, ▲, ■, ✖) or 1.0 (○, △, □, +). As a control, other tumors were irradiated with gamma-rays alone (⧅). ●, ○ without neutron capture drug; ▲, △ with BPA administration; ■, □ with BSH administration; ✖, + with Omniscan administration. Only mean values are shown, to avoid confusion.

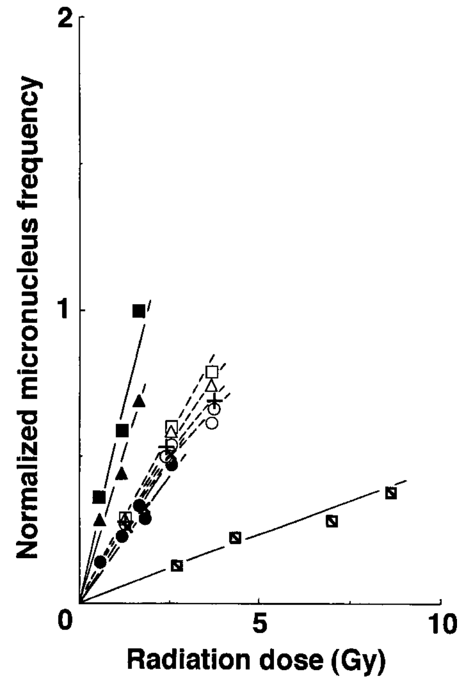

Fig. 2. Dose-response curves of normalized MN frequency as a function of radiation dose for quiescent tumor cell populations. Tumors were irradiated using neutrons with a Cd ratio of 148 (●, ▲, ■, ✖) or 1.0 (○, △, □, +). As a control, other tumors were irradiated with gamma-rays alone (⧅). ●, ○ without neutron capture drug; ▲, △ with BPA administration; ■, □ with BSH administration; ✖, + with Omniscan administration. Only mean values are shown, to avoid confusion.

than those for total tumor cells ($P<0.05$). Moreover, in each cell population, the values for low Cd ratio neutrons were larger than those for high Cd ratio neutrons, although not significantly so.

With neutron capture compounds, the normalized MN frequency for each cell population was increased. This increase in the normalized MN frequency was marked when high Cd ratio neutrons were used. To assess the effects of these compounds on the MN frequency in total and Q cell populations, the enhancement ratio (ER) was calculated at various normalized MN frequencies using the mean values of the data given in Figs. 1 and 2 (Table V). In general, the values of ERs for total cells and high Cd ratio neutrons tended to be larger than those for Q cells and low Cd ratio neutrons, respectively. With neutrons of each Cd ratio, in total tumor cells, the ER values for BPA administration were larger than those for any other drug. In contrast, in Q cells, the values for BSH were largest. This tendency was clearly observed in higher Cd ratio neutrons. Omniscan only slightly increased MN frequency both in total and Q cells, compared with the irradiation alone, without drugs.

DISCUSSION

The effects of cytochalasin-B on chromosome damage in irradiated cells have not been completely elucidated. However, a close relationship between cell survival and MN frequency for SCC VII tumor cells obtained using the cytochalasin-B method has been reported.[11] Namely, the linear correlation between surviving fraction and MN frequency demonstrated that tumor cell sensitivity can be expressed in terms of the MN frequency instead of surviving fraction. Additionally, Ono *et al.*[13] showed that more than 90% of SCC VII tumor cells divided at least once following neutron capture irradiation and that the probability of completing the first post-treatment mitosis was almost equivalent in SCC VII cells regardless of neutron capture compound administration. Consequently, the sensitivity of tumor cells to neutron capture irradiation was thought to be reflected reasonably well by their MN frequency instead of by their loss of clonogenicity.

The advantages of high linear energy transfer (LET) neutron irradiation include: 1) greater ability to damage

hypoxic cells; 2) lesser ability to repair sublethal and potentially lethal radiation-induced damage; and 3) less variation in radiation sensitivity relative to the cell cycle.[14] We showed previously that Q cell populations have a larger percentage of hypoxic cells than the total cell population.[5] In this study, the differences in radiosensitivity between total tumor and Q cells were markedly reduced by neutron irradiation (Table III). It follows that oxygenated and hypoxic rcells in SCC VII solid tumors have almost the same radiosensitivity to neutrons and that the difference in sensitivity elative to the cell cycle can be decreased with neutrons. However, there was no apparent relationship between the difference in sensitivity between total and Q cells and the value of neutron Cd ratio. Therefore, high LET neutrons, irrespective of neutron Cd ratio, should be applied to reduce the difference in sensitivity between total and Q cells.

The values of RBE for Q cells were markedly larger than those for total cells. This was mainly because the difference in gamma-ray sensitivity between total and Q cells was much greater than that in sensitivity to neutrons (Table III). Further, in both cell populations, the RBE values for low Cd ratio neutrons tended to be larger than those for high Cd

Table III. Dose Modifying Factors[a] for Quiescent Cells Relative to the Total Tumor Cell Populations

Normalized MN freq.[b]	Neutrons		Gamma-rays
	Cd[c]:148	Cd:1.0	
0.5	1.5	1.6	—
0.35	1.5	1.5	2.4
0.25	1.5	1.6	2.4

a) Radiation dose required to obtain each normalized micronucleus frequency in quiescent cells/radiation dose required to obtain each normalized micronucleus frequency in total tumor cells.
b) Normalized micronucleus frequency, micronucleus frequency minus the micronucleus frequency in unirradiated tumors
c) Cadmium ratio.

Table IV. Relative Biological Effectiveness[a] in Total Tumor and Quiescent Cell Populations

Cell fraction	Normalized MN freq.[b]	Neutrons	
		Cd[c]:148	Cd:1.0
Total tumor cells	0.75	—	2.9
	0.5	2.4	2.9
	0.35	2.4	2.8
	0.25	2.3	2.9
Quiescent tumor cells	0.35	3.7	4.5
	0.25	3.6	4.4

a) Radiation dose required to obtain each normalized micronucleus frequency with gamma-rays/radiation dose required to obtain each normalized micronucleus frequency with neutrons.
b) Normalized micronucleus frequency, micronucleus frequency minus the micronucleus frequency in unirradiated tumors.
c) Cadmium ratio.

Table V. The Effects of Neutron Capture Drugs on the Dose Modifying Factor[a]

Cell fraction	Cadmium ratio	Normalized MN freq.[b]	Drugs		
			BSH[c]	BPA[d]	Omniscan[e]
Total tumor cells	Cd ratio: 148	0.5	3.7	5.4	1.2
		0.35	3.6	5.1	1.2
		0.25	3.7	5.5	1.2
	Cd ratio: 1.0	1.0	1.2	1.3	1.1
		0.75	1.2	1.4	1.1
		0.5	1.2	1.3	1.1
		0.35	1.2	1.4	1.1
		0.25	1.2	1.3	1.1
Quiescent tumor cells	Cd ratio: 148	0.5	3.2	2.4	1.2
		0.35	3.3	2.4	1.2
		0.25	3.4	2.4	1.2
	Cd ratio: 1.0	0.5	1.2	1.1	1.1
		0.35	1.2	1.1	1.1
		0.25	1.2	1.2	1.1

a) Radiation dose required to obtain each normalized micronucleus frequency without ^{10}B-compound/radiation dose required to obtain each normalized micronucleus frequency with ^{10}B-compound.
b) Normalized micronucleus frequency, micronucleus frequency minus the micronucleus frequency in unirradiated tumors.
c) Sodium borocaptate-^{10}B.
d) *dl-p*-Boronophenylalanine-^{10}B.
e) Gadodiamide hydrate.

ratio neutrons. This might have been partially because the low Cd ratio neutrons applied here included a higher proportion of fast neutrons (Table I), which include a larger proportion of 24 keV neutrons that are considered to induce chromosome abberations at high efficiency.[15, 16] The values of RBE for total cells themselves were similar to those in other reports.[17, 18] However, to our knowledge, this is the first report concerning RBE for Q cells *in vivo* related to neutron Cd ratio in neutron irradiation.

^{10}B-compounds increased the sensitivity of both Q and total cell populations, especially that of total cells. Namely, the difference in sensitivity between total and Q cells was widened with ^{10}B-compound. This means that i) Q cells cannot take up ^{10}B as efficiently as P cells, or ii) the sensitivity of Q cells is intrinsically lower than that of P cells even if ^{10}B is homogeneously distributed throughout P and Q cells. As shown in our previous study using fast neutrons[19] and Table III, high LET neutron irradiation alone without compounds markedly reduces the difference in radiosensitivity between P and Q cells. Thus, the homogeneous ^{10}B distribution through P and Q cells is also supposed to decrease the difference in sensitivity between total and Q cells. Therefore, it is thought that Q cells cannot take up ^{10}B as efficiently as P cells. Further, the sensitivity of Q cells treated with BPA was lower than that in those treated with BSH, although the average ^{10}B concentration for BPA administration (10.7±2.8 μg/g) was almost the same as that for BSH (11.2±1.2 μg/g) in the total tumor population. This tendency was clearly observed with higher Cd ratio neutrons which include a larger proportion of thermal neutrons. In other words, when BPA was used, ^{10}B could be distributed to total tumor cells as a whole as well as when BSH was used. However, less ^{10}B could be localized into Q cells than when BSH was administered. This indicates that the distribution of ^{10}B, from BPA, in tumor cells is more dependent on the ^{10}B uptake ability of the tumor cells than that from BSH. When BSH was used, the ER values for total cells were almost the same as those for Q cells. That is, the distribution of ^{10}B from BSH is more dependent on the diffusion of the drug than that from BPA.

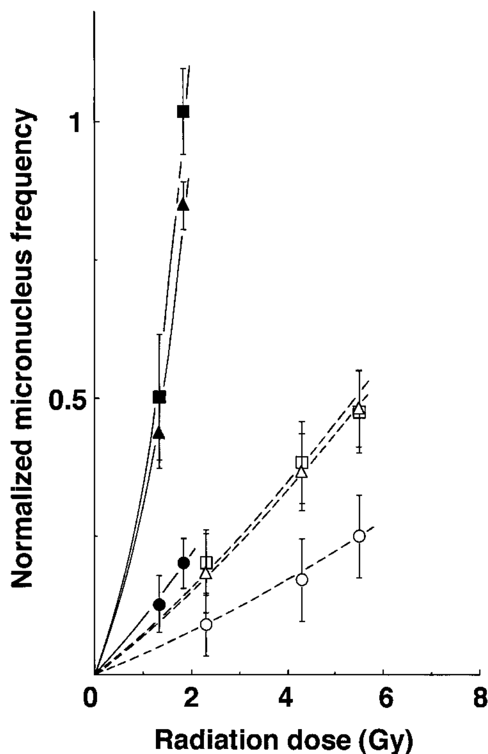

Fig. 3. Dose-response curves of normalized MN frequency as a function of radiation dose for cultured SCC VII tumor cells. Cultured cells were irradiated using neutrons with a Cd ratio of 148 (●, ▲, ■) or 1.0 (○, △, □). ●, ○ without neutron capture drug; ▲, △ with boric acid-^{10}B at the ^{10}B concentration of 1 m*M*; ■, □ with Omniscan at the ^{157}Gd concentration of 3 m*M*. Bars represent SDs.

Our previous *in vitro* experiment using single cell suspensions of the SCC VII tumor cell line and the two kinds of Cd ratio (148 and 1.0) neutrons showed that Omniscan with 3.0 m*M* ^{157}Gd could increase the sensitivity of cultured tumor cells to the same extent as boric acid enriched with 1.0 m*M* ^{10}B (Fig.3). Similarly to that experiment, the tumors treated with ^{10}B-compounds or Omniscan also contained 1.0 m*M* ^{10}B or 3.0 m*M* ^{157}Gd. Nevertheless, both in total and Q cells, Omniscan could not increase MN frequency as markedly as any other ^{10}B-compound and could only slightly increase MN frequency, compared with the irradiation alone, without drugs. This is partly because the interactions between thermal neutrons and ^{157}Gd atoms result in emission of photons and electrons with broad energy levels up to 7.9 MeV, which are considered to be mostly of low LET.[20] It was reported that tumor cell growth was suppressed by NCT with Gd due to the long ranges of emitted gamma-rays and electrons from ^{157}Gd even if Gd was present only around tumor cells.[21] Another factor is that Omniscan is too water-soluble (partition coefficient (octanol/water, pH = 2.0–10.0) ≤ 1.2×10^{-4}) to be accumulated in tumor tissue for a sufficient period during neutron irradiation. Recently, to accumulate Gd at high concentrations in tumor tissue, microcapsules containing Gd for intra-arterial injection have been designed and synthesized.[22] The water-soluble Gd-containing contrast media for MRI was, however, not suitable as a neutron capture compound in NCT.

Solid tumors, especially human tumors, are thought to contain a high proportion of Q cells.[23] The presence of these cells is probably due, in part, to hypoxia and the depletion of nutrition in the tumor core, and this is another consequence of poor vascular supply.[4] Therefore, Q cells may have shown higher MN frequencies at 0 Gy than total cells (Table II). It has been reported that Q cells have lower radiosensitivity than P cells in solid tumors *in vivo*.[4, 23] As

Table VI. The Effects of Neutron Capture Drugs on Relative Biological Effectiveness[a)]

Cell fraction	Cadmium ratio	Normalized MN freq.[b)]	Drugs		
			BSH[c)]	BPA[d)]	Omniscan[e)]
Total tumor cells	Cd ratio: 148	0.5	8.9	12.9	2.6
		0.35	8.6	12.2	2.6
		0.25	8.5	12.6	2.5
	Cd ratio: 1.0	0.75	3.5	4.1	3.2
		0.5	3.5	3.8	3.2
		0.35	3.4	3.9	3.1
		0.25	3.5	3.8	3.2
Quiescent tumor cells	Cd ratio: 148	0.35	12.2	8.9	4.1
		0.25	12.2	8.7	4.0
	Cd ratio: 1.0	0.35	5.4	5.0	5.0
		0.25	5.3	5.3	4.8

a) Radiation dose required to obtain each normalized micronucleus frequency with gamma-rays/radiation dose required to obtain each normalized micronucleus frequency with neutrons combined with neutron capture drug administration.
b) Normalized micronucleus frequency, micronucleus frequency minus the micronucleus frequency in unirradiated tumors.
c) Sodium borocaptate-^{10}B.
d) *dl*-*p*-Boronophenylalanine-^{10}B.
e) Gadodiamide hydrate.

was also shown in this study, Q cells have significantly lower radiosensitivity to gamma-rays than the total cell population within solid tumors *in vivo* (Table III). This means that more Q cells can survive after radiotherapy than P cells. Consequently, the control of Q cells, some of which still have clonogenicity,[4)] is thought to influence greatly the outcome of anticancer radiotherapy. To evaluate the usefulness of neutron capture compound administration before neutron irradiation, the RBE values for total and Q cells compared with gamma-ray irradiation were calculated by multiplying the values shown in Table V by those in Table IV (Table VI). This table shows that the use of BSH combined with high Cd ratio neutron irradiation is effective in the control of radioresistant Q cells and that BPA with high Cd ratio neutrons is effective for controlling the total cells. From the viewpoint of tumor curability, the combination of BSH and BPA may be useful in NCT using high Cd ratio neutrons.[24)]

The characterization of Q cells in solid tumors and their sensitivity to various treatments has been greatly hampered by the lack of adequate systems to identify Q cells and obtain them in large homogeneous populations. The Q cell assay method used in this study is expected to be useful for determining the sensitivity of Q cell populations in solid tumors to ionizing radiation. Using this method, we plan to investigate the responses of Q cells to various anticancer treatments. Further, in some tumors, it is now evident that apoptosis correlates well with the curability of the tumors.[25, 26)] We also plan to assess the effectiveness of neutron capture reaction in inducing apoptosis.

ACKNOWLEDGMENTS

This study was supported in part by Grants-in-Aid for Cancer Research (04857111, 05807076, 08877139, 09255229, 09670931) from the Ministry of Education, Science, Sports, and Culture, Japan.

(Received July 17, 1997 / Revised September 10, 1997 / 2nd Revised October 9, 1997 / Accepted October 16, 1997)

REFERENCES

1) Barth, R. F., Soloway, A. H. and Brugger, R. M. Boron neutron capture therapy of brain tumors: past history, current status, and future potential. *Cancer Invest.*, **14**, 534–550 (1996).
2) Greenwood, R. C., Reich, C. W., Baader, H. A., Koch, H. R., Breitig, D., Schult, O. W. B., Fogelberg, B., Backlin, A., Mampe, W., von Egidy, T. and Schreckenbach, K. Collective and two-quasiparticle states in ^{158}Gd observed through study of radioactive neutron capture in ^{157}Gd. *Nucl. Phys.*, **A304**, 327–428 (1978).
3) Kagehira, K., Sakurai, Y., Kobayashi, T., Kanda, K. and Akine, Y. Physical dose evaluation on gadolinium neutron capture therapy. *Annu. Rep. Res. React. Inst. Kyoto Univ.*, **27**, 42–56 (1994).

4) Jackson, R. C. The problem of the quiescent cancer cell. *Enzyme Regul*., **29**, 27–46 (1989).
5) Masunaga, S., Ono, K. and Abe, M. A method for selective measurement of the radiosensitivity of quiescent cells in solid tumors—combination of immunofluorescence staining to BrdU and micronucleus assay. *Radiat. Res*., **125**, 243–247 (1991).
6) Kanda, K. An overview: radiation sources, beam quality, dosimetry and spectroscopy in neutron capture therapy. *Strahlenther. Onkol*., **165**, 67–69 (1989).
7) Sakurai, Y., Kobayashi, T., Kanda, K. and Ono, K. The irradiation characteristics of the remodelled KUR heavy water facility. *In* "Kyoto University Research Reactor Institute Progress Report 1995," ed. Res. React. Inst. Kyoto Univ., p. 199 (1996). Kumatori, Osaka.
8) Caswell, R. S., Coyne, J. J. and Randolph, M. L. Kerma factors for neutron energies below 30 MeV. *Radiat. Res*., **83**, 217–254 (1980).
9) Snyder, W. S., Cook, M. J., Nasset, E. S., Karhausen, L. R., Parry Howells, G. and Tipton, I. Gross and elemental content of reference man. *In* "Report of the Task Group on Reference Man," ed. W. S. Snyder, pp. 273–324 (1975). Pergamon Press, Oxford, UK.
10) Kobayashi, T., Kanda, K. and Mishima, Y. *In situ* measurement on 10B concentrations and absorbed dose estimations in human malignant melanoma treated by BNCT. *Strahlenther. Onkol*., **165**, 104–106 (1989).
11) Ono, K., Wandl, E. O., Tsutsui, K., Sasai, K. and Abe, M. The correlation between cell survival curve and dose response curve of micronucleus (MN) frequency. *Strahlenther. Onkol*., **165**, 824–827 (1989).
12) Mitchell, J., Morstyn, G., Russo, A., Kinsella, T., Fornance, A., MacPherson, S. and Glatstein, E. Differing sensitivity to fluorescent light in Chinese hamster cells containing equally incorporated quantities of BUdR versus IUdR. *Int. J. Radiat. Oncol. Biol. Phys*., **10**, 1447–1451 (1984).
13) Ono, K., Masunaga, S., Kinashi, Y., Takagaki, M., Akaboshi, M., Kobayashi, T. and Akuta, K. Radiobiological evidence suggesting heterogeneous microdistribution of boron compounds in tumors: its relation to quiescent cell population and tumor cure in neutron capture therapy. *Int. J. Radiat. Oncol. Biol. Phys*., **34**, 1081–1086 (1996).
14) Wambersie, A., Bewley, D. K. and Lalanne, C. M. Prospects for the application of fast neutrons in cancer therapy. Radiobiological bases and survey of the clinical data. *Bull. Cancer Paris*, **73**, 546–561 (1986).
15) Aghamohammadi, S. Z., Goodhead, D. T. and Savage, J. R. Production of chromosome aberrations, micronuclei, and sister-chromatid exchanges by 24-keV epithermal neutrons in human G0 lymphocytes. *Mutat. Res*., **211**, 225–230 (1989).
16) Hill, A. J., Morgan, G. R. and Newman, S. M. Cell survival measurements in an argon, aluminium and sulphur filtered neutron beam: a comparison with 24 keV neutrons and relevance to boron neutron capture therapy. *Br. J. Radiol*., **67**, 1008–1016 (1994).
17) Ujeno, Y. Physical modification of thermal neutron-induced biological effects. *In* "Modification of Radiosensitivity in Cancer Treatment," ed. T. Sugahara, pp. 477–486 (1984). Academic Press, Tokyo.
18) Utsumi, H. The radiation biology of boron neutron capture therapy. *Nucl. Sci. Appl*., **4**, 325–333 (1991).
19) Masunaga, S., Ono, K., Akuta, K., Akaboshi, M., Ando, K. and Koike, S. The response of quiescent cell populations in murine solid tumors to irradiation with fast neutrons. *Acta Oncol*., **33**, 813–817 (1994).
20) Akine, Y., Tokita, N., Matsumoto, T., Oyama, H., Egawa, S. and Aizawa, O. Radiation effect of gadolinium-neutron capture reactions on the survival of Chinese hamster cells. *Strahlenther. Onkol*., **166**, 831–833 (1990).
21) Akine, Y., Tokita, N., Tokuuye, K., Satoh, M., Kobayashi, T. and Kanda, K. Electron-equivalent dose for the effect of gadolinium neutron capture therapy on the growth of subcutaneously-inoculated Ehrlich tumor cells in mice. *Jpn. J. Clin. Oncol*., **23**, 145–148 (1993).
22) Fukumori, Y., Ichikawa, H., Tokumitsu, H., Miyamoto, M., Jono, K., Kanamori, R., Akine, Y. and Tokita, N. Design and preparation of ethyl cellulose microcapsules of gadopentetate dimeglumine for neutron-capture therapy using the Wurster process. *Chem. Pharm. Bull.* (*Tokyo*), **41**, 1144–1148 (1993).
23) Wallen, C. A., Ridinger, D. N. and Dethlefsen, L. A. Heterogeneity of X-ray cytotoxicity in proliferating and quiescent murine mammary carcinoma cells. *Cancer Res*., **45**, 3064–3069 (1985).
24) Ono, K., Masunaga, S., Kinashi, Y., Takagaki, M. and Kobayashi, T. Combined effect of BPA and BSH in boron neutron capture therapy for murine tumors. *In* "Kyoto University Research Reactor Institute Progress Report 1995," ed. Res. React. Inst. Kyoto Univ., p. 183 (1996). Kumatori, Osaka.
25) Wheeler, J. A., Stephans, L. C., Tornos, C., Eifel, P. J., Ang, K. K., Milas, L., Allen, P. K. and Meyn, R. E., Jr. ASTRO Research Fellowship: apoptosis as a predictor of tumor response to radiation in stage IB cervical carcinoma. *Int. J. Radiat. Oncol. Biol. Phys*., **32**, 1487–1493 (1995).
26) Denmeade, S. R., Lin, X. S. and Isaacs, J. T. Role of programmed (apoptotic) cell death during the progression and therapy for prostate cancer. *Prostate*, **28**, 251–265 (1996).