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ENHANCED THERAPEUTIC EFFECT ON MURINE MELANOMA AND ANGIOSARCOMA CELLS BY BORON NEUTRON CAPTURE THERAPY USING A BORONATED METALLOPORPHYRIN

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SYNOPSIS

We have already achieved successful treatment of several human patients with malignant melanoma by boron neutron capture therapy (BNCT) using $^{10}B_1$-paraboronophenylalanine ($^{10}B_1$-BPA•HCl). In this study we used a new compound, a manganese boronated protoporphyrin (Mn-^{10}BOPP), and compared it to $^{10}B_1$-BPA•HCl with respect to uptake in murine melanoma and angiosarcoma cells as well as to their cell killing effect. ^{10}B uptake was measured in a new method, and the new compound was much more incorporated into both cells than $^{10}B_1$-BPA•HCl. Furthermore,melanoma and angiosarcoma cells preincubated with the new compound were 15 to 20 times more efficiently killed by BNCT than cells preincubated with $^{10}B_1$-BPA•HCl.

SUMMARY

We have already achieved successful treatment of several human patients with malignant melanoma by boron neutron capture therapy (BNCT) using $^{10}B_1$-paraboronophenylalanine ($^{10}B_1$-BPA).

In this communication we used a new compound, a manganese boronated protoporphyrin (Mn-^{10}BOPP), and compared it to $^{10}B_1$-BPA•HCl with respect to uptake in murine melanoma and angiosarcoma cells as well as to their respective cell killing effects using BNCT.

Mn-^{10}BOPP was found to be 3 to 10 times more effectively taken up by melanoma cells and angiosarcoma cells than $^{10}B_1$-BPA•HCl when the content of ^{10}B was measured by the new method, inductively coupled plasma-mass spectrometry (ICP-MS). In addition, melanoma cells and

angiosarcoma cells preincubated with Mn-^{10}BOPP were 15 to 20 times more efficiently killed by BNCT than cells preincubated with $^{10}B_1$-BPA•HCl. These results suggest that Mn-^{10}BOPP is a suitable compound for BNCT of not only malignant melanoma but also angiosarcoma.

INTRODUCTION

The successful application of boron neutron capture therapy (BNCT) to malignant melanoma and other solid tumors requires that a boron carrier meets two principal requirements: [1] high accumulation of ^{10}B in the target cells (approximately 20 ppm or more), and [2] sufficiently rapid clearance from blood and normal tissue surrounding the tumor to permit a tumor-to-blood and tumor-to-normal tissue ratio in excess of one for brain tumor, and aprroximately four for melanoma. The ideal compound would be one in which tumor boron levels and these ratios were simultaneously high. We have already experimentally achieved both requirements and have completed successful treatment of several malignant melanoma patients by BNCT using $^{10}B_1$-paraboronophenylalanine ($^{10}B_1$-BPA) [10]. Coderre and coworkers have extended the application of BPA to successful BNCT treatment of intracerebral gliomas in rats [2] and an intraocular melanoma in rabbits [11].

The application of $^{10}B_1$-BPA in the BNCT, however, is complicated by several factors. The weight percentage of boron in BPA is only about 5%, so that the administration of large total doses of the compound is necessary. As the free amino acid, BPA has limited solubility at physiological pH which makes it difficult to administer by intravenous or intraperitoneal injection. Orally administered BPA (intragastrically) produces therapeutically useful

levels of boron in animals with model tumors, but the compound is not well-absorbed by the gastrointestinal tract. Finally, the dihydroxy boryl group of BPA is not hydrolytically stable under physiologic conditions. Thus, in the present study, we chose to compare $^{10}B_1$-BPA•HCl and a new boronated metalloporphyrin with respect to their relative abilities to be taken up by murine melanoma and angiosarcoma cells and their cytotoxic effects in the presence of thermal neutrons.

BOPP is a tetracarborane carboxylate ester of deuteroporphyrin IX 2,4-bis-glycol having a molecular weight of 1390 with naturally abundant boron and 1357 in the ^{10}B-enriched form [8]. The corresponding weight percentages of boron are 31% and 29.5%, respectively. It is highly water-soluble and is readily converted to metalloporphyrins by standard methods [1]. Like most free base porphyrins, aqueous solutions of BOPP are highly photosensitive and need to be handled under low light conditions to avoid phototoxic effect on cells and photodecomposition in dilute solutions.

BOPP has been found to be a highly tumor-selective agent in intracerebral glioma model systems where a tumor-to-normal brain ratio of 400 : 1 have been obtained with simultaneous tumor porphyrin concentrations corresponding to 65 ppm boron [4]. Mn-BOPP has been shown to be a highly effective MRI contrast enhancement agent in an intracerebral glioma model in rats, and has been suggested as a dual purpose agent for the diagnosis and treatment of this disease [6]. The present study was undertaken in order to directly compare Mn-^{10}BOPP and $^{10}B_1$-BPA•HCl.

MATERIALS AND METHODS

Compounds

Mn-^{10}BOPP was prepared from the BOPP free acid by the method of Huang L.R. et al. [6] using ^{10}B-enriched (>95%) BOPP. The dipotassium salt form was used in the present syudies. ^{10}B-enriched BPA•HCl was prepared by the method of Snyder [12].

Determination of Cell Number in One Gram Wet Melanoma Tissue

Protein was extracted by the Schmidt-Thannhanser-Schneider method from 20 mg of B-16 melanoma tissue obtained from a subcutaneously transplanted syngenic C57BL/6 mouse, and its protein content per gram (TP) was measured by Lowry method [9]. The protein content of 4×10^6 cultured B-16 melanoma cells (CP) was also measured similarly. The cell number in 20 mg of wet melanoma tissue was calculated by the following equation: $4 \times 10^6 \times TP / CP$.

Mesurement of ^{10}B of Cultured Cells by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

Semiconfluent B-16 melanoma cells and F-2 angiosarcoma cells [13] cultured in 30 cm^2-dishes were incubated with $^{10}B_1$-BPA•HCl (16.3 μg ^{10}B / ml) or Mn-^{10}BOPP (10 μg ^{10}B / ml) in complete cell culture medium for 0 to 12 hours in an incubator. For the measurement of ^{10}B accumulation, mechanically collected cells were counted, digested with 0.3 ml of 60 % perchloric acid and 0.6 ml of 30 % hydrogen peroxide, and decomposed for 4 hours at 70 °C. After membrane filtration, the ^{10}B contents of the samples were assayed by ICP-MS[5].

Evaluation of Tumor Cell Killing by BNCT Using Mn-^{10}BOPP or $^{10}B_1$-BPA•HCl

B-16 melanoma cells and F-2 angiosarcoma cells were incubated with culture medium containing $^{10}B_1$-BPA•HCl (16.3 μg ^{10}B / ml) or Mn-^{10}BOPP (10 μg ^{10}B / ml) for 20 hours. The cells were trypsinized and 1.0 x 10^5 cells were suspended in normal medium in teflon tubes. The teflon tubes were placed at varying distances from the bismuth face of the neutron source at the Kyoto University Reactor (KUR) in order to obtain various neutron fluences (0.2-1.2 x 10^{13} n / cm^2) [7]. The total time from the beginning of removal of the cells from the incubation medium to the end of irradiation ranged from 6 to 7 hours. After irradiation, cells were seeded in 30 cm^2-dishes and cultured for 10 days. Cells were then fixed and stained for colony counting in order to measure the surviving fraction. Each experimental point on the dose-survival curve was obtained from the means of at least three measurements.

RESULTS AND DISCUSSION

The cell number of cultured B-16 melanoma cells equivalent to 1 gram wet weight was found to be 1.73 x 10^8, which is approximately one sixth of the widely used conversion value, 1 x 10^9. We assumed that the F-2 angiosarcoma cells have the same weight.

B-16 melanoma cells were accumulated $^{10}B_1$-BPA•HCl and Mn-^{10}BOPP in a time dependent manner, and Mn-^{10}BOPP accumulated more rapidly in these cells than $^{10}B_1$-BPA•HCl (Fig. 1a). The mean concentration of ^{10}B in B-16 cells incubated with Mn-^{10}BOPP was 298.3 ppm after 2-hour incubation, whereas the boron content of cells exposed to $^{10}B_1$-BPA•HCl

was 73.9 ppm after 2 hours. F-2 sarcoma cells incorporated 17 times more Mn-^{10}BOPP (531.4 ppm) than $^{10}B_1$-BPA•HCl (32.2 ppm) within an hour (Fig. 1b). After a 12-hour incubation, B-16 melanoma cells took up more than 300 ppm of ^{10}B. Mn-^{10}BOPP at the concentration of 10 μg ^{10}B / ml was not cytotoxic (data not shown).

The reasons for the much higher accumulation of Mn-^{10}BOPP than $^{10}B_1$-BPA•HCl in both cell lines are not clear, but may be related to the low density lipoprotein (LDL) receptor status of transformed cells. In order to maintain vigorous proliferative activity, many tumor cells have an upregulated LDL receptor activity. Porphyrins are known to be bound and transported by lipoproteins, especially LDL, and may be incorporated in cells through receptor mediated endocytosis [3]. Previous studies in our laboratory have established that BOPP binds extensively to LDL. Presumably so does Mn-BOPP.

When B-16 and F-2 cells were exposed to thermal neutron after preincubation with $^{10}B_1$-BPA•HCl or Mn-^{10}BOPP ,both compounds induced extensive cell kill (Fig.2a and 2b). However , the magnitude of the cytotoxic effect was much greater in the cells treated with Mn-^{10}BOPP than with $^{10}B_1$-BPA•HCl. At the thermal neutron fluence of 1×10^{13} n / cm^2, both cell lines were killed 15 to 20 times more efficiently by BNCT using Mn-^{10}BOPP than $^{10}B_1$-BPA•HCl. The D_0 value, representing the radiation dose required to reduce cell survival by a factor of 1/e on the linear portion of the survival curve, was obtained from the slope of each dose-survival curve (Table 1). The D_0 of melanoma or angiosarcoma cells preincubated with $^{10}B_1$-BPA•HCl was one and half times more than that of cells preincubated with Mn-^{10}BOPP, indicating that cells exposed to the boronated porphyrin at the doses used are significantly more sensitized to thermal neutrons.

BOPP itself has been shown to localize into the mitochondrial fraction of the cytoplasmic contents [4], and the pharmacokinetic profiles of BOPP and Mn-BOPP appear to be similar. BPA also appears to be actively transported into the cytoplasm of some cancer cells through an aromatic amino acid uptake system. It is not, however, a substrate for the tyrosinase enzyme found in melanotic melanoma cells, and its fate after internalization is not known.

Although extrapolation of in vitro data to in vivo circumstances is difficult, these data are suggestive that Mn-^{10}BOPP might be a suitable compound for BNCT of melanoma and angiosarcoma in vivo. The cellular boron contents obtained in these experiments with Mn-^{10}BOPP are the highest reported for any candidate compound described in the literatures. When adjusted on a boron content per mg boron administerd are exceptionally high. Moreover, the presence of the paramagnetic Mn (III) in the porphyrin core suggests a possible usefulness as a magnetic resonance contrast enhancement agent and raises a possibility that Mn-^{10}BOPP could be used simultaneously as a diagnostic and therapeutic agent for melanoma and angiosarcoma.

Table 1. D_0 value of thermal neutron irradiated cells preincubated with Mn-^{10}BOPP or $^{10}B_1$-BPA•HCl

	D_0 ($\times 10^{12}$ n/cm^2)	
	B-16 melanoma	F-2 angiosarcoma
Boron free control	2.9	3.2
$^{10}B_1$-BPA•HCl	2.1	2.2
Mn-^{10}BOPP	1.4	1.5

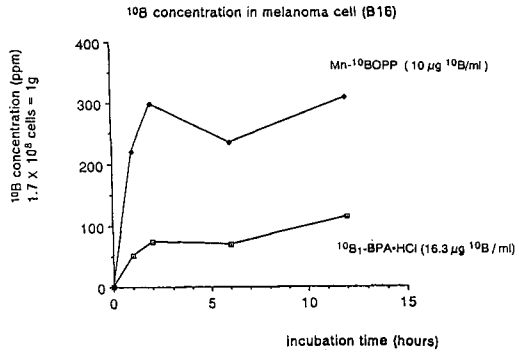

Fig. 1a

B-16 melanoma cells were incubated with $^{10}B_1$-BPA•HCl (16.3 μg^{10}B/ml) or Mn-^{10}BOPP (10 μg^{10}B/ml) in complete cell culture medium for 0 to 12 hours in a CO_2 incubator. ^{10}B contents of the samples were assayed by ICP-MS. B-16 melanoma cells incorporated both compounds in a time dependent manner, and Mn-^{10}BOPP was accumulated more rapidly than $^{10}B_1$-BPA•HCl.

Each value represents a mean of three experimental results.

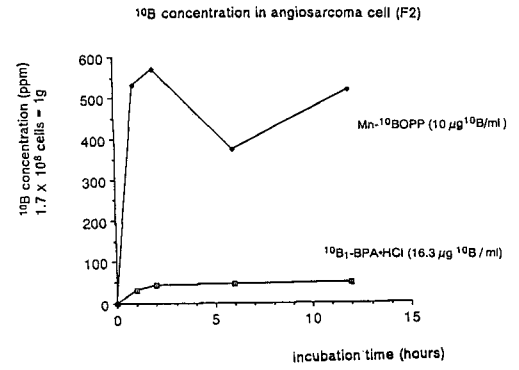

Fig. 1b

F-2 angiosarcoma cells were incubated in the same way as B-16 melanoma cells. And ^{10}B contents of the samples were assayed by ICP-MS. F-2 angiosarcoma cells incorporated 17 times more Mn-^{10}BOPP (531.4 ppm) than $^{10}B_1$-BPA•HCl (32.2 ppm) at an hour incubation.

dose survival curves of B-16

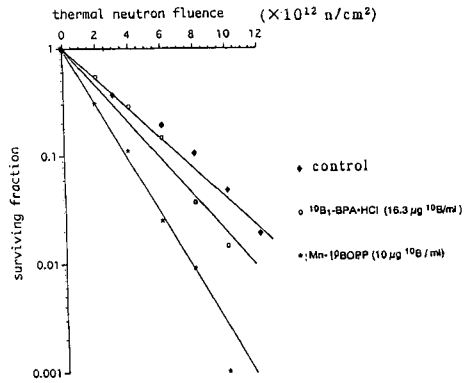

Fig. 2a

B-16 melanoma cells were incubated with culture medium containing $^{10}B_1$-BPA·HCl (16.3 $\mu g^{10}B/ml$) or Mn-^{10}BOPP (10 $\mu g^{10}B/ml$) for 20 hours. The cells were exposed to various neutron fluences (0.2-1.2 $\times 10^{12} n/cm^2$). After irradiation, various numbers of cells were cultured for 10 days to obtain colonies. Cells were then fixed and stained for colony counting in order to determine the surviving fraction. Both compounds induced extensive cell kill after thermal neutron irradiation. However, the cytotoxic effect was much greater in B-16 cells treated with Mn-^{10}BOPP than with $^{10}B_1$-BPA·HCl. The mean plating efficiency for B-16 melanoma cells without irradiation was apparantly 80%.

dose survival curves of F-2

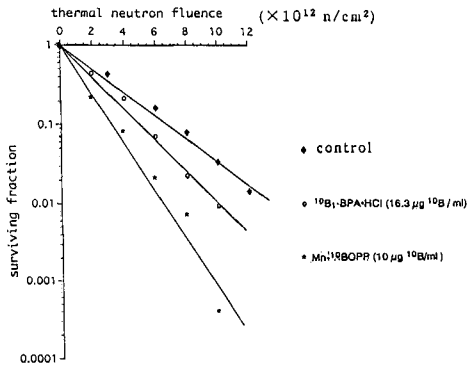

Fig. 2b

F-2 angiosarcoma cells were incubated with culture medium containing $^{10}B_1$-BPA·HCl (16.3 $\mu g^{10}B/ml$) or Mn-^{10}BOPP (10 $\mu g^{10}B/ml$) for 20 hours. And the cells were obtained various neutron fluences (0.2-1.2$\times 10^{12} n/cm^2$). After irradiation, F-2 cells were cultured, fixed and stained in the same way of B-16 cells. The cell killing effect was much greater in F-2 cells treated with Mn-^{10}BOPP than with $^{10}B_1$-BPA·HCl. The mean plating efficiency for F-2 cells without irradiation was about 85%.

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