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Studies for the application of boron neutron capture therapy to the treatment of differentiated thyroid cancer

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ABSTRACT

The aim of these studies was to evaluate the possibility of treating differentiated thyroid cancer by BNCT. These carcinomas are well controlled with surgery followed by therapy with ^{131}I; however, some patients do not respond to this treatment. BPA uptake was analyzed both *in vitro* and in nude mice implanted with cell lines of differentiated thyroid carcinoma. The boron intracellular concentration in the different cell lines and the biodistribution studies showed the selectivity of the BPA uptake by this kind of tumor.

1. Introduction

Boron neutron capture therapy (BNCT) is a binary experimental treatment for cancer, which requires the selective uptake of ^{10}B by the tumor in an amount of at least 15–25 ppm. When these requisites are reached, the tumor area is irradiated with an appropriate neutron beam (epithermal or thermal), depending on the tumor depth. The isotope ^{11}B, product of this neutron capture reaction, promptly releases an alpha particle and a ^{7}Li nucleus (< 10 μm of range), in principle causing the tumor cell death without significant damage to the surrounding normal tissues (Coderre and Morris, 1999). Clinical trials of BNCT using p-boronophenylalanine (^{10}BPA) or sodium borocaptate (^{10}BSH) are being performed for high grade gliomas, cutaneous melanomas and for tumors of head and neck (Yamamoto et al., 2008; Aihara et al., 2006; Kato et al., 2004; Liberman et al., 2004).

BNCT has been proposed as an option for the treatment of undifferentiated thyroid cancer (UTC) (Pisarev et al., 2005). We have shown that cells of human undifferentiated thyroid carcinoma have a selective uptake of ^{10}BPA, both *in vitro* and after being transplanted to NIH nude mice (Dagrosa et al., 2002). Also the application of BNCT using ^{10}BPA alone and combined with the tetrakis-carborane carboxylate ester of 2,4-bis-(α, β-dihydroxythyl)-deutero-porphyrin IX (BOPP) showed a complete halt of tumor growth in 100% of the animals and a complete histological cure in 50% and 100% of the mice bearing tumors smaller than 50 mm^3, respectively (Dagrosa et al., 2003, 2007).

However at diagnosis more than 80% of patients with thyroid neoplasms present differentiated carcinoma (DTC). The differentiated forms, such as papillary or follicular carcinomas, are in general of a relatively benign prognosis. Most of them still have a normal uptake of iodine and therefore surgical thyroidectomy is completed with a therapeutic dose of ^{131}I. In many cases these forms are well controlled and complete remission is obtained, but in other instances the prognosis is not so good. Some of the recurrent differentiated forms have lost their capability to concentrate radioiodine and therefore cannot be treated with therapeutic dose of this halogen (Gilliland et al., 1997; Are and Shaha, 2006). BNCT can be an alternative treatment for these patients. In these studies we analyzed the ^{10}BPA uptake *in vitro* and in nude mice implanted with a human cell line of differentiated thyroid carcinoma. The results show that it is possible to treat this tumor by BNCT.

2. Materials and methods

2.1. Cell lines

The human cell lines of follicular (WRO) and papillary (TPC-1) carcinomas were grown in RPMI and modified DMEM medium, respectively. The cell line of rat normal thyroid, FRTL-5, used as normal control, was cultured in DMEM/F12. The three culture mediums were supplemented with 10% of FBS and grown under 5% CO_2 and at 37 °C.

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2.2. Iodide uptake

Cells were washed and incubated at 37 °C with 0.5 μCi carrier free Na ^{125}I (17 Ci/mg) New England Nuclear and 1 μM sodium iodide in medium RPMI 1640. After 30 min, cells were centrifuged at 1500*g* for 5 min, washed rapidly with ice-cold buffered HBSS plus 10^{-6} M KI and its radioactivity was measured. Iodide uptake was expressed as total radioactivity and referred to protein content according to Lowry method (Lowry et al., 1951).

2.3. Preparation of ^{10}BPA solution

BPA was purchased from Boron Biochemical (New Raleigh, MO, USA). The solution of ^{10}BPA–fructose was prepared at a concentration of 30 mg ^{10}BPA/mL (0.14 M) as it was described by Coderre et al. (1998). Briefly, ^{10}BPA (95% atom % ^{10}B-enriched) L-isomer, was combined in water with a 10% molar excess of fructose, the pH was adjusted to 9.5–10 with NaOH, the mixture was stirred until all solids dissolved, and the pH was then readjusted to 7.4 with HCl.

2.4. BPA cellular uptake

Exponentially growing cells were incubated with ^{10}BPA at a final concentration of 6.93 mM (50 μg ^{10}B/mL). After 2 h of incubation, cells were washed, scrapped in PBS and centrifuged at 900*g*. The pellets were digested for 2 h at 60 °C with 0.15 mL of a 1:1 mixture of concentrated nitric and sulfuric acids. Dilution to 1.0 mL was performed with 0.65 mL of a 5% aqueous solution of Triton X-100 (v/v) and 0.2 mL of a solution containing 25 μg/mL Sr and 0.5 μg/mL Y as internal standards. The ^{10}BPA uptake was measured by inductively coupled plasma optical emission spectroscopy (ICP-OES) and the boron content was referred to the total proteins. Analytical and internal standard lines (in nm) were as follows: B: 249.677; Sr: 232.235; and Y: 371.029. Matrix-matched standard solutions containing the internal standard elements and boron between 0.1 and 10.0 μg/mL were employed for daily calibration.

2.5. Animal model

NIH nude mice, (body weight 20–25 g) were implanted in the back right flank with 1.5×10^6 of WRO cells. The animals were bred and maintained under aseptic conditions. For the *in vivo* studies a total number of 43 animals were used.

2.6. Tumor growth measurement

The size of the tumors was measured with a caliper two or three times a week and the volume was calculated according to the following formula: $A^2 \times B/2$ (where A is the width and B is the length) (Lee et al., 1988). After 25 days, when the tumors had a size between 45 and 200 mm^3, the mice were used for biodistribution studies.

2.7. Biodistribution studies

To evaluate the ^{10}BPA uptake, animals were injected with the boron compound at a dose of 350 mg/Kg b.w (ip) and sacrificed at 0.5, 1, 2.0 and 3.0 h post administration. Digestion of tissue samples, with masses ranging between 10 and 50 mg, was carried out and boron measurements were performed as described above.

2.8. Histological studies

Histological preparations were made from the tumor and the surrounding skin. These tissues were fixed with 10% buffered-formol pH 7.0, included in paraffin and stained with hematoxylin and eosin (HE).

2.9. Statistical analysis

Data were analyzed according to analysis of variance (ANOVA) and to Bonferroni multiple comparisons. Values were considered significant when $p < 0.05$.

3. Results and discussion

Fig. 1 shows a low radioactive iodide uptake for both tumor cell lines (TPC-1 and WRO). However the thyroid normal cells (FRTL5) stimulated by TSH (hormone that regulate the growth and the thyroid function) and without stimulation have an important iodide uptake ($p < 0.001$). The iodide uptake ratio for FRTL5/WRO was 305 and for FRTL5/TPC-1 was 76.35. This behavior resembles the situation observed in patients with recurrent differentiated thyroid cancer.

On the contrary the boron intracellular concentration ratios between WRO and TPC-1 vs. FRTL-5 were 5.44 and 2.97, showing the selectivity of the ^{10}BPA uptake by both tumor cell lines (Fig. 2). The larger uptake by tumor cells may reflect the more active metabolism. In this regard, analysis of kinetics in patient with either melanoma or glioblastoma using ^{18}F-labeled BPA has shown that the net incorporation rate for tumor was about four times that of normal brain (Mishima et al., 1997). The mechanism

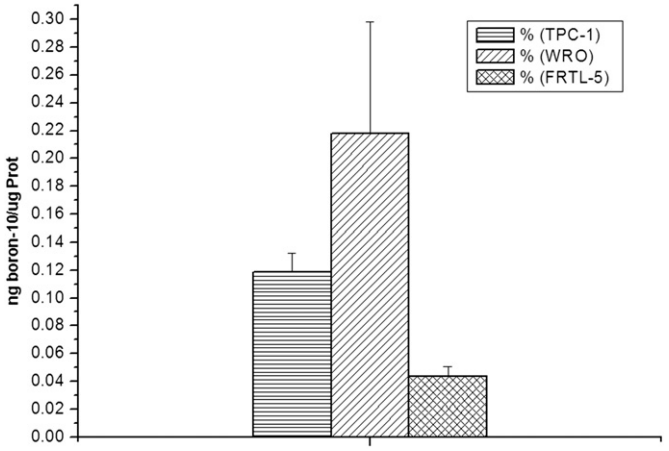

Fig. 1. ^{125}I uptake by thyroid normal cells (FRTL-5) and two human cell lines of differentiated thyroid carcinoma (WRO and TPC-1).

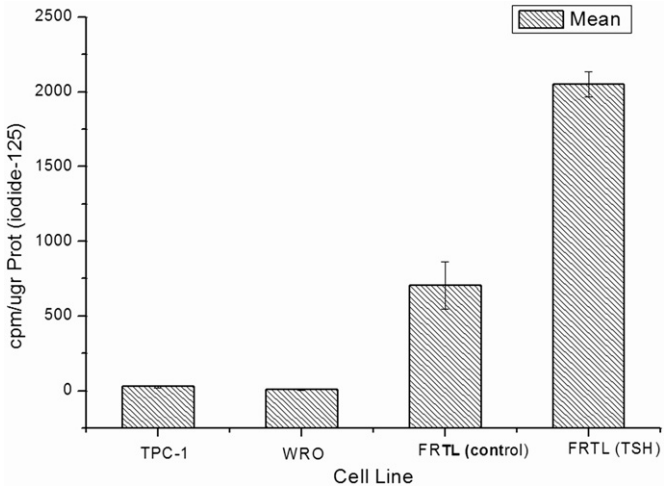

Fig. 2. ^{10}BPA uptake (50 ppm ^{10}B) by normal thyroid cells (FRTL5) and cell lines of DTC (WRO and TPC1) after 2 h of incubation. The ^{10}BPA uptake ratios were WRO/FRTL-5 = 5.44; TPC-1/FRTL-5 = 2.97.

of transport of ^{10}BPA was analyzed in the glioblastoma cell line GS-9L, and in the fibroblast cell line V79. The results supported the hypothesis that ^{10}BPA is transported by the L-amino acid transport system (Wittig et al., 2000).

The follicular carcinoma (WRO) cells in a number of 1.5×10^6 were transplanted into NIH-nude mice. Fig. 3 shows the tumor growth as a function of the time. The tumors started to grow at 25 day post implantation in an exponential way, reaching values between 45 and 255 mm^3. All the animals implanted with WRO cells developed tumors.

Histological studies showed a tumor with follicular structure, big cells, and nucleus of different sizes. These tumors have large areas of necrosis (Fig. 4).

Table 1 and Fig. 5 show the results of the ^{10}BPA biodistribution at a dose of 350 mg/Kg b.w. in NIH nude mice. A peak in the boron uptake by the tumor after 2 h post administration can be observed. At this time the ^{10}B concentrations/g tissue expressed as mean value ± SEM were: tumor 21.98 ± 6.04, blood 6.38 ± 0.25, thyroid 7.83 ± 1.29, liver 7.59 ± 4.24, spleen 9.23 ± 3.16, lung 6.66 ± 1.13, kidney 14.92 ± 2.39, surrounding skin 11.24 ± 4.22 and distal skin 10.28 ± 2.28. The ratios between tumor and normal surrounding tissues (blood and distal skin) were 3.44 and 2.14, respectively. Only the kidney, where ^{10}BPA is excreted, had values of boron greater than other tissues. Time-course studies showed that the peak of ^{10}BPA concentration in the tumor cells is dependent on the amount of compound injected (Dagrosa et al., 2002).

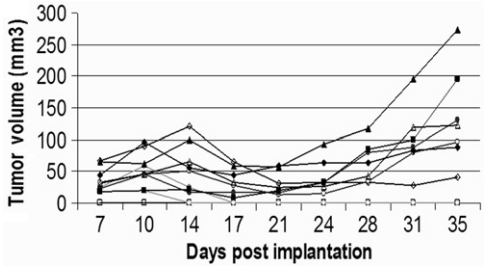

Fig. 3. Tumor growth in nude NIH nude mice (6–8 weeks) implanted with 1.5×10^6 of WRO cells.

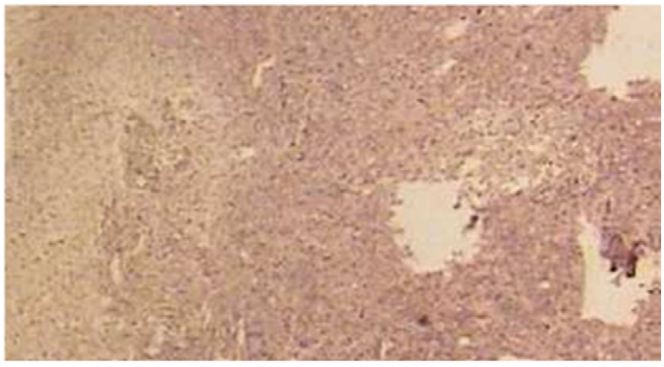

Fig. 4. Histology of follicular thyroid carcinoma, 2 weeks after transplantation of the WRO cells into nude mice. 10X (HE staining).

Table 1
Biodistribution of ^{10}BPA in NIH nude mice. ^{10}B concentrations were determined by ICP-OES in various organs of the NIH nude mice. The animals were sacrificed at different times (0.5, 1, 2.0 and 3.0 h) after the injection of 350 mg/kg body weight ^{10}BPA. Values are the average+SEM of 5–9 animals from two different experiments. Each value is expressed as μg ^{10}B/g tissue (ppm).

	0.5 h	1 h	2 h	3 h
Tumor	16.01 ± 3.54	19.81 ± 4.53	21.98 ± 6.04	16.07 ± 5.36
Surrounding skin	11.92 ± 4.71	11.89 ± 3.44	11.24 ± 4.22	8.56 ± 2.88
Distal skin	10.07 ± 2.47	10.67 ± 1.97	10.28 ± 2.28	6.21 ± 1.13
Lung	15.84 ± 5.19	9.6 ± 1.65	6.66 ± 1.13	5.76 ± 0.49
Kidney	40.95 ± 6.18	28.58 ± 4.29	14.92 ± 2.39	13.3 ± 2.06
Spleen	20.23 ± 4.38	11.68 ± 2.38	9.23 ± 3.16	8–6 ± 3.04
Thyroid	12.61 ± 4.09	13.05 ± 1.37	7.83 ± 1.29	5.23 ± 0.89
Liver	11.83 ± 2.45	8.19 ± 1.56	7.59 ± 4.24	5.41 ± 0.33
Blood	10.05 ± 1.98	7.53 ± 1.28	6.38 ± 0.25	4.78 ± 0.29

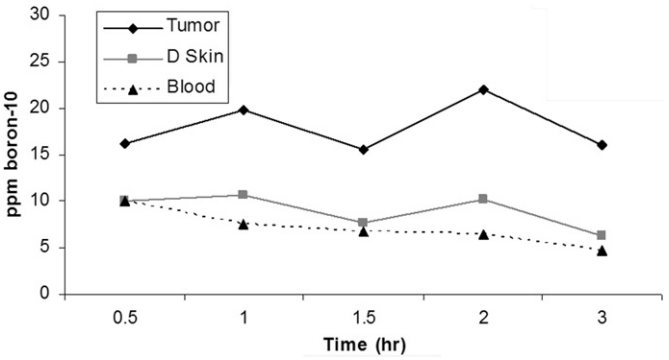

Fig. 5. Biodistribution of ^{10}BPA (350 mg/Kg b.w) in tumor, distal skin and blood. Each point is the average of 5–9 animals ± SEM. The ratios were: T/S= 3.44 ($p < 0.01$); T/DS=2.14 ($p < 0.01$) (2 h).

0This tumor has a selective uptake of ^{10}BPA and it could be treated by BNCT. Recurrent differentiated thyroid carcinomas are very aggressive and they cannot be treated by the therapeutic dose of radioiodide. Also they are resistant to both chemo and radiotherapy. These forms have a fatal outcome in rather a short time after their diagnosis as a consequence new treatments that are being explored in order to offer these patients a better quality of life. One patient with a relapsed papillary thyroid cancer was treated by BNCT in Japan in September 2003. No adverse effects were observed (Kato et al., 2006).

4. Conclusions

The studied cell lines of thyroid carcinoma showed a biological behavior similar to that observed in patients. The ^{10}BPA uptake in CDT cells (follicular and papillary forms) is similar to that observed previously in UTC cells. This is the first step towards the possible application of BNCT to the treatment of relapsed cancer which, as mentioned above, becomes more aggressive and resistant to the traditional forms of therapy.

Conflict of interest

We declare that there is no conflict of interest in this paper.

Acknowledgments

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