Journal of Neuro-Oncology **62:** 145–156, 2003.

Tissue uptake of BSH in patients with glioblastoma in the EORTC 11961 phase I BNCT trial

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Key words: BNCT, glioblastoma, BSH tissue uptake, BSH toxicity

Summary

Purpose: The uptake of the boron compound $Na_2B_{12}H_{10}$-SH (BSH) in tumor and normal tissues was investigated in the frame of the EORTC phase I trial 'Postoperative treatment of glioblastoma with BNCT at the Petten Irradiation Facility' (protocol 11961).

Methods and Materials: The boron concentration in blood, tumor, normal brain, dura, muscle, skin and bone was detected using inductively coupled plasma-atomic emission spectroscopy in 13 evaluable patients. In a first group of 10 patients 100 mg BSH/kg bodyweight (BW) were administered; a second group of 3 patients received 22.9 mg BSH/kg BW. The toxicity due to BSH was evaluated.

Results: The average boron concentration in the tumor was 19.9 ± 9.1 ppm (1 standard deviation (SD)) in the high dose group and 9.8 ± 3.3 ppm in the low dose group, the tumor/blood ratios were 0.6 ± 0.2 and 0.9 ± 0.2, respectively. The highest boron uptake has been detected in the dura, very low uptake was found in the bone, the cerebro-spinal fluid and especially in the brain (brain/blood ratio 0.2 ± 0.02 and 0.4 ± 0.2). No toxicity was detected except flush-like symptoms in 2 cases during a BSH infusion at a much higher speed than prescribed.

Conclusion: BSH proved to be safe for clinical application at a dose of 100 mg BSH/kg infused and at a dose rate of 1 mg/kg/min. The study underlines the importance of a further investigation of BSH uptake in order to obtain enough data for significant statistical analysis. The boron concentration in blood seems to be a quite reliable parameter to predict the boron concentration in other tissues.

Introduction

Boron neutron capture therapy (BNCT) is a radiotherapy modality based on the nuclear reaction that occurs when the non-radioactive isotope boron-10 captures a thermal neutron. The released high LET radiation consists of an alpha particle and a Li-nucleus with a range in tissue of approximately 10 μm. The effects of this irradiation with high biological effectiveness are therefore limited to approximately one cell diameter. In principle, one event liberates enough energy to kill one cell. However, neutron capture therapy is clinically attractive only if a sufficiently high thermal neutron fluence can be delivered to the target volume and if a sufficiently high concentration of ^{10}B can be obtained in the tumor, with relatively

low concentrations in the surrounding healthy tissue. Knowledge about tissue uptake of boron compounds available for BNCT is therefore of major importance.

Up to now, only two agents are available for clinical investigation: the boron cluster sodium mercaptoundecahydrododecaborate $Na_2{}^{10}B_{12}H_{10}SH$, referred to as BSH [1,2] and the amino acid analogue paraboronophenylalanine $C_9H_{12}{}^{10}BNO_4$ (BPA) [3,4].

Both compounds are being used in actual clinical trials for glioblastoma and melanomas [5,6]. BSH was first used in 1968 by Hatanaka and more recently by Nakagawa [7,8] in Japan to treat malignant glioma in individual patients. It was also used in several studies to evaluate its pharmacokinetic properties and the biodistribution of ^{10}B as delivered by BSH. BSH is also under evaluation in the EORTC phase I trial 11961 'Postoperative Treatment of Glioblastoma with BNCT at the Petten Irradiation Facility' (EORTC: European Organisation for the Research and Treatment of Cancer) [9,10]. As part of this clinical trial an investigation of the uptake of BSH in the tumor and in surrounding tissues has been performed. The results of this research are presented in this paper.

Methods and patients

The clinical trial

The phase I protocol EORTC 11961 is a dose finding trial. Its primary aim is to determine systemic and local toxicity of boron neutron capture therapy (BNCT) with the compound BSH, after craniotomy with gross total tumor resection in patients with glioblastoma multiforme. It investigates the qualitative and quantitative dose-limiting toxicity and maximum tolerated dose of this regimen and aims to establish the maximum tolerated radiation dose of BNCT to healthy tissues under well-defined conditions of cranial irradiation. Cohorts of patients are irradiated in 4 fractions at the same radiation dose level with epithermal neutrons at the European Commission's research reactor High Flux Reactor (HFR) in Petten (NL). The average ^{10}B-concentration in blood over the 4 fractions is 30 ppm for all patients. The increase of radiation dose from one cohort to the next is obtained by increasing the irradiation time. Systemic toxicity due to the drug is investigated up to 30 days after BSH infusion and is reported using the Common Toxicity Criteria of the National Cancer Institute (NCI CTC). For the grading of adverse events during the first 90 days after radiotherapy, NCI CTC and EORTC/RTOG (RTOG: Radiation Therapy Oncology Group) early radiation toxicity criteria are applied. After this observation period, EORTC/RTOG late radiation morbidity scales are used in combination with SOMA [9].

The secondary goal of the study is to evaluate the ^{10}B-uptake in tumor and healthy tissues. Therefore, protocol 11961 includes a tissue uptake study for the first cohort of patients included in the trial.

The multi-center study includes 5 participating patient referral centers from 4 different countries: the Departments of Neurosurgery at the Klinikum Großhadern in Munich (D), the CHU Hôpital Pasteur, Nice (F), the Zentralkrankenhaus Bremen (D), the Karl-Franzens-University, Graz (A) and the Vrije Universiteit Amsterdam (NL). The study center is the Department of Radiotherapy of the University Essen (D). In order to assure the quality of the work to be performed, it is necessary to create a very specialized organization and contractual structure [14]. Furthermore, due to the fact that a new drug, a new radiation beam and a new facility are used, special efforts were made on quality management. A detailed description of all procedures is provided in written form (Standard Operating Procedures, SOP's) to all participating institutes. The infrastructure and preparation are controlled and the study procedures are agreed and practiced in detail during an initiation site visit. In addition to the case report forms, special study documents are provided (source document forms, submission forms) to achieve a high degree of unambiguity and uniformity [9].

Tissue sampling

In the first cohort of patients, the tissue uptake of boron after infusion of BSH was investigated. Prior to surgery, BSH was administered at the same amount as foreseen for the first fraction of irradiation: 100 mg/per kg BW. The time interval between infusion and operation was the same as that intended for BNCT: 12–14 h. During surgery, tissues were collected from areas where exposure to the neutron beam during the planned BNCT was expected. The sampling was performed in a way so as not to compromise the planned surgical intervention, which led to the collection of different numbers of specimens in each individual patient. The following tissues were collected during surgery: tumor, non-tumor brain tissue, dura mater, calvarium, muscle, skin and cerebrospinal fluid (CSF). Whenever possible, samples were acquired from a number of different locations in the

tumor. Blood was collected at the same time as tissue sampling. The content of boron in blood and tissues was measured by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) at the Nuclear Research and consultancy Group (NRG) Petten [12,13].

Analytical procedure

For the ICP-AES, the 249.773 nm emission line was chosen to measure boron. For a 4 ml sample, the detection limit, defined as the mean value of the background +3 SDs, was between 0.001 and 0.015 ppm. This allowed the detection of between 0.04 and 0.6 ppm of boron in tissue sample with the size of 1 g. All measurements were done in triplicate with a coefficient of variation of less than 2%. Internal standard samples were measured with prompt gamma ray spectroscopy.

Boron compound

Boron-10 enriched (>95%) BSH is prepared as a chemically defined compound supplied in vials, each containing 1000 mg of the substance. Supplying companies have to produce the compound according to a drug master file, in which a written procedure has to be followed for preparation and quality control of the final product and its intermediates. The material is imported into The Netherlands by the Pharmacy Department of the University/Academic Hospital 'Vrije Universiteit' (AZVU) in Amsterdam [15]. Prior to use in humans, strict quality checks are performed for each batch following standard operating procedures (SOP). In the laboratory of the AZVU pharmacy, the following quality control checks are performed:

1. Identification of the study medication: Test for the presence of sodium and identification of the product by infrared absorption spectrophotometry.
2. Tests on purity of BSH in its ionic form $[(B_{12}H_{10}SH)_2]^-$ as well as absence of its oxidation products $[(B_{24}H_{22}SH_2)_4^-$ and $(B_{24}H_{22}S_2O)_4]^-$: This is tested by high-pressure liquid chromatography. The material is defined to meet the requirements if the total of the oxidation products is less than 2%.
3. Absence of bacterial endotoxins (pyrogens): This is tested by Limulus Amoebocyte Lysate test. The material is defined to meet the requirements if it contains less than 0.025 IE pyrogens/mg BSH.
4. Assays of the study medication: These tests are carried out at NRG in Petten using ICP-AES and prompt gamma spectroscopy under the auspices of the AZVU pharmacists according to written procedures:
   - BSH content: The material is defined to meet the requirements if the total amount of BSH in each tested vial is 95–105% of the stated value.
   - Degree of enrichment: The material is defined to meet the requirements if the degree of enrichment is >95%.

The responsibility for the quality control and for the release of the material for clinical use is delegated to two independent pharmacists. If the batch meets all requirements, the pharmacist releases it for clinical use with an expiry date one year after initial testing. After labeling conforming to good clinical practice, the material is sent to the collaborating centers together with a certificate of analysis provided by the Pharmacy Department of the AZVU and a declaration that the material is suitable for clinical use. Before administration to the patient, the drug is dissolved under aseptic conditions. A vial containing 1 g BSH is dissolved in 10 ml distilled water. This solution is sterile-filtered using a 0.2 μm sterile filter and added to a 0.9% NaCl infusion to obtain a total volume of 500 ml to contain 100 mg BSH/kg BW of a given patient. It is always required to use the total amount of one vial containing 1 g BSH. Rounding should be to lower values (e.g. for a patient weighing 75 kg, seven vials of 1 g each are used). The material remains stable at room temperature for at least 6 h and is administered within that time interval. The infusion rate is defined to be 1 mg BSH/kg BW/minute. If feasible, the infusion should be given through a central venous catheter.

The patients

Fourteen patients (12 males and 2 females), in the age range between 51 and 74 years (mean: 61.2 years), weighing between 56 and 109 kg with operable glioblastoma multiforme (GBM) as suspected by CT and MRI images were admitted to the first cohort of the study after informed consent was given (Table 1). Central pathology review at the German Brain Tumour Reference Centre in Bonn (Prof. Wiestler, Bonn, Germany) revealed GBM (WHO grade IV) in 11 patients and Gliosarcoma (WHO grade IV) in 3 patients from tumor samples received from the respective neurosurgeon. Of these, 13 patients could be evaluated from the point of view of tissue boron uptake. None of the patients had reduced kidney or liver function, and

Table 1. Demographic data of treated patients and BSH dose

Patient	Gender	Age	Performance status (Karnofsky)	Dose of BSH (mg/kg)
101	M	74	80	103.9
102	M	52	80	101.4
103	M	73	90	100.0
104	M	59	70	98.2
105	M	56	80	90.9
106	F	63	90	95.2
107	F	57	90	89.7
108	M	71	100	92.1
109	M	60	80	94.6
110	M	58	100	89.9
111	M	64	100	20.4
112	M	51	90	21.1
113	M	51	100	8.8
114	M	68	100	27.3

none suffered from any other malignant disease. The first 10 patients were infused 14 h prior to surgery with 100 mg BSH/kg BW (range 89.7–103.9), at an infusion rate of 1 mg BSH/kg BW/min according to the protocol plan. The total amount of BSH infused varied between 5000 and 9000 mg. Due to a temporary lack of an adequate amount of BSH, three patients received only a total dose of 2000 mg BSH each, corresponding to 20.4–28.2 mg BSH/kg BW (22.9 mg BSH/kg BW on average) infused at the same rate. One patient (#113) received only 8.8 mg BSH/kg BW and was excluded from this evaluation.

For the patients who received 100 mg BSH/kg BW, the boron concentration in tissues was evaluated normalized to 100 mg BSH/kg BW, assuming that the amount of boron in the tumor and in other tissues increases proportionally with the dose of BSH administered [16,17]. For the 3 patients who received only a total dose of 2 g BSH, the boron concentration was evaluated normalized to 22.9 mg BSH/kg BW, being the average dose of BSH given to these 3 patients (range: 20.4–27.3 mg BSH/kg BW).

Results

The boron contents in all tumor and tissue specimens of the individual patients are presented in Tables 2 and 3. Due to the limited number of samples available, a SD cannot be calculated for all values.

Tumor

The maximum number of tumor samples taken from any patient was 5, for 5 patients only one sample from the operated tumor was made available. In some patients only one sample could be taken in order not to compromise the surgical procedure. A total average value over all samples has been evaluated. The average boron-concentration in tumor was 19.8 ± 9.3 ppm (1SD) for the patients who received 100 mg BSH/kg BW and 9.8 ± 3.3 ppm for the 3 patients who received only 22.9 mg BSH/kg BW. The boron content in tumor tissue varies considerably from one patient to another. In the cases in which several samples from one single tumor were available, a highly heterogeneous boron distribution was observed in the individual tumor samples. The average tumor to blood ratio at the time point of the tissue sampling (12 h after the BSH infusion) was 0.6 and 0.9, respectively, in the 2 groups of patients. The average tumor to blood ratio was with the exception of one patient always less than 1. It is also just as significant to note that there was considerable intratumoral variability in boron uptake.

Brain

The 'brain' tissue investigated was tissue adjacent to the tumor, which had to be removed in order to operate the tumor. No brain tissue at a distance from the tumor site was taken. The average boron concentration in the brain tissue adjacent to the tumor was 6.6 ppm in the patients who received 100 mg BSH/kg BW and 3.5 ppm in patients who received 22.9 mg BSH/kg BW. In all patients, the average boron concentration in brain was lower than in blood, tumor and all other tissues investigated, only in bone the boron concentration was even lower than in brain. The tumor to brain ratio for the two groups of patients was 2.9 and 3.0, respectively, the brain to blood ratio was 0.2 and 0.4.

Additional normal tissues

There was a clear difference in boron content between the different tissues investigated. The boron concentration in the cranial bone was always very low: 5.3 ± 2.7 ppm. The bone to blood ratio was 0.2 ± 0.1 in 3 patients who received the high dose of BSH and 0.1 in the patient who received the lower dose.

Very high boron uptake was detected in skin with an average boron concentration of 42.1 and 11.8 ppm in the second dose group and dura (51.2 and 21.1 ppm). The SD was also very high. In muscle tissue a lower boron uptake was detected (24.3 and 7.8 ppm).

Cerebro-spinal fluid was collected for boron analysis from 2 patients only: one sample had to be excluded

Table 2. Boron concentration measurements in normal tissue and tumor specimens. The table indicates the absolute BSH dose that was applied to the individual patients. The boron measurements in all tissues and blood were normalized to 100 mg/kg BW

	Patient no.										Average 101–110	Standard deviation(s)
	101	102	103	104	105	106	107	108	109	110		
mg BSH/kg	103.9	101.4	100.0	98.2	90.9	95.2	89.7	92.1	94.6	89.9	95.6	5.1
Normalization factor to 100 mg BSH/kg BW	0.96	0.99	1.00	1.02	1.10	1.05	1.11	1.09	1.06	1.11	1.05	0.05
Boron concentration in normal tissues and in tumor (ppm)												
Blood	48.9	33.7	35.5	28.7	43.0	27.5	29.6	25.1	26.5	42.9	34.2	8.2
CSF				30.6*				2.3				
Skin		21.6				15.2	137.0	19.5	14.5	44.7	42.1	47.8
Muscle		11.1	35.2	15.0	15.1		62.8	12.8	20.5	21.5	24.3	17.3
Bone			8.6	3.7			6.4			2.6	5.3	2.7
Dura		38.2	42.2	17.8	30.7	16.2	145.8	104.1	31.4	34.2	51.2	43.9
Brain	9.4						4.7	4.1		8.2	6.6	2.6
Tumor	27.3	17.8	16.5	20.6	38.9	7.5	12.5	21.5	12.7	12.8		
Tumor	41.8		18.2	18.8	24.2	11.7	3.0		26.2			
Tumor	23.9		25.4	13.1								
Tumor			21.1									
Tumor			21.6									
Tumor (average)	31.0		20.6	17.5	31.6	9.6	7.7		19.5	12.8	19.9	9.1
SD	9.5		3.4	3.9								
Ratios of boron between different tissues												
Tumor/blood	0.6	0.5	0.6	0.6	0.7	0.3	0.3	0.9	0.7	0.3	0.6	0.2
Tumor/brain	3.3						1.7	5.2		1.6	2.9	1.7
Skin/blood		0.6				0.6	4.6	0.8	0.5	1.0	1.4	1.6
Bone/blood			0.2	0.1			0.2			0.1	0.2	0.1
Dura/blood		1.1		0.6	0.7	0.6	4.9	4.2	1.2	0.8	1.8	1.7
Brain/blood	0.2						0.2	0.2		0.2	0.2	0.02

*Value not representative. Sample was contaminated with blood.

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