Quantitative Neutron Capture Radiography for Studying the Biodistribution of Tumor-seeking Boron-containing Compounds

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ABSTRACT

Biodistribution of two compounds presently considered for use in neutron capture therapy has been studied in mice carrying a transplantable Harding-Passey melanoma. A method is described by which quantitative assessment can be made of the boron distribution in whole-body sections of such animals. An α-particle-sensitive film is placed in close contact with a freeze-dried section of an animal and exposed to neutrons. The tracks visible after etching are analyzed optoelectronically in fields of 0.6 x 0.6 mm² and compared to standards of boron homogeneously distributed in liver homogenates.

The dynamic range of this method is about two orders of magnitude in concentration, with a lower detection limit of 0.1 to 0.01 ppm ¹⁰⁶B, depending on the rate of induction of spurious tracks by fast neutrons present in the neutron beam chosen.

In a transplantable Harding-Passey melanoma in mice, it was found that the sulfhydryl boron hydride Na₂Bi₂H₉SH presently used for therapy of glioblastoma clears blood, muscle, and brain very rapidly. Its accumulation in tumors was persistent for more than three days. A higher tumor accumulation was observed with its disulfide, which has been suggested for neutron capture therapy.

For both compounds, a marked heterogeneity of boron distribution within one tumor was found.

INTRODUCTION

Boron neutron capture therapy is based on the irradiation of tumors with slow neutrons after the accumulation of boron through physiological pathways and clearance from surrounding normal tissues. ¹⁰⁶B captures neutrons and produces α- and ³⁷Li particles through the reaction ¹⁰⁶(B(n,α)⁷Li). This reaction is very efficient in cell killing (1). Fairchild and Bond (2) have calculated the necessary accumulation of boron and found that ¹⁰⁶B concentrations between 3 and 50 ppm (i.e., μg ¹⁰⁶B/g tissue) have to be present in order to deliver a therapeutically useful dose.

Presently, boron neutron capture therapy is applied clinically in Japan in the treatment of glioblastoma, using the sulfhydryl boron hydride (BSH) as boron carrier (3). Other potential carriers include BSSB, the disulfide of the above BSH (4), porphyrins (5), antibodies (6), and amino acids (7).

The development of new compounds leads eventually to the need to analyze for ppm amounts of boron in tissues. Originally, this was done by colorimetric assay of boric acid after ashing (8). This procedure is tedious and, in addition, presents problems in the analysis of borane cages such as BSH (4).

Received 12/31/86; revised 6/29/87; accepted 7/13/87.

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¹ This work has been supported by grants from the Deutsche Forschungsgemeinschaft, Fonds der Chemischen Industrie, and the North Atlantic Treaty Organization (D. G., H. H.); by the Swedish Natural Science Research Council and the Swedish Cancer Society (B. L., L. G., G. E.); and by the U.S. Department of Energy contract no. DE-AC02-76CM00016 (P. S., D. S., R. G. F.).

² The abbreviations used are: BSH, Na₂Bi₂H₉SH; BSSB, Na₂Bi₂H₉S₂; QNCR, quantitative neutron capture radiography; MRR, Brookhaven National Laboratory Medical Research Reactor.

The use of prompt-γ spectroscopy (9, 10) allows the analysis of ¹⁰⁶B in ppm amounts without the need for chemical conversion. It requires, however, several μg of ¹⁰⁶B. It is thus not easily adapted to distribution studies in small animals, such as mice. In addition, it requires expensive equipment and extensive access to a neutron beam. Finally, most exploratory chemical studies are carried out with natural boron, where ¹⁰⁶B represents only 20%, ¹¹B being the predominant isotope. The sensitivity of the prompt-γ technique is thereby reduced to such a degree that therapeutic amounts of boron would have to be present before a reliable analysis can be obtained.

For boron in solution, the use of track-etch detectors for quantitative analysis has been proposed (10–12). With this technique, very small amounts of ¹⁰⁶B (in the order of ng) can be detected through the ¹⁰⁶(B(n,γ)⁷Li) reaction. However, this technique is less suited for distribution studies because it would require homogenization of minute tissue samples and thus is too laborious for routine use.

Previously, track-etch detectors have been used for visualization of boron in sections of tissue and animals (13–17). It has been difficult to obtain absolute concentrations, however. Amano and Sweet (13), Matsuoka et al. (14), and Abe et al. (17) have used standards of boron in gelatin exposed separately or together with the sample. Because different amounts of water were present in sample and standard, corrections had to be made to obtain reliable absolute concentration data. Since individual tracks were counted microscopically, the dynamic range of the method was limited when tracks overlapped.

In this paper, we describe a technique to determine quantitatively the amount of boron in tissue and whole-body sections with track-etch detectors. We have used tissue as a matrix for boron standards, thereby eliminating the need for corrections. The standards were present in every section. By comparing the area of all holes (rather than counting individual tracks) in a small field with the area of holes in a standard with known concentrations of boron, boron can be determined quantitatively. With this technique, it is possible to quantify the amount of ¹⁰⁶B present in organs or parts of organs, down to concentrations of 0.01 ppm.

MATERIALS AND METHODS

Chemicals. BSH and its disulfide BSSB (both ¹⁰⁶B enriched) were prepared from their cesium salts (purchased from Callery Co., Callery, PA) as described before (4).

Track-Etch Detector. The cellulose nitrate film LR 115 Type I from Kodak Pathé (Paris, France) was used. This detector was etched in 10% NaOH at 60°C for 45 min, i.e., until the holes penetrated the detector layer and were clearly visible under the microscope.

Tumor Model. The tumor model used was the transplantable Harding-Passey melanoma. Its morphology and histology was as described in (18). No gross vascular space is present. The central volume of large tumors contains many melanophores (18) and appears to be partly necrotic.

Preparation of Whole-Body Mouse Sections for Analysis. BSH and...
BSSB were dissolved in water at a concentration of 1.4 mg 10B/ml. Of these solutions, 0.5 ml (about 35 µg 10B/g body weight) was injected i.p. into BALB/c mice carrying a s.c. transplanted Harding-Passay melanoma. The animals were sacrificed at the times indicated.

Animals were killed by ether and embedded in 1.57% carboxymethyl cellulose together with standards prepared as described below. After freezing, sagittal sections of 50-µm thickness were cut and mounted on tape, as described by Ullberg (19). The sections were freeze-dried. The dried sections were squeezed (by means of foam rubber or a vacuum bag) against the detector. The sections were not stained. Staining should be avoided before neutron exposure in order to prevent boron leaching and diffusion.

Standards. Boron standards were prepared by thoroughly mixing chicken liver homogenates with 10% by weight boracic acid solutions of the appropriate concentrations (between 0.01 and 100 ppm boron of natural abundance). The slurries were introduced into dialysis bags with an internal diameter of 6 mm. The frozen bags were cut into appropriate lengths and stuck into the carboxymethyl cellulose near the animals just prior to freezing the block. Concentrations used were usually 100, 30, 10, 3, 1, 0.3, 0.1, 0.03, and 0.01 ppm boron (natural abundance) together with a tissue blank.

Standards prepared with polyacrylamide gels of approximately the same concentration of solids as the tissue were not suitable; the drying of the gels led to shrinking and warping of the section of the standard. Although this was not attempted here, the standards could be shaped for easier identification. In our experiments, we identified the standards by their positions.

Tapes. Several tapes from 3M (Minneapolis, MN, obtained directly or through LKB) and one tape from Tuck Industries, Inc. (New Rochelle, NY) were used for mounting the sections. Tapes of 3M type 810 with the batch numbers 1G1, 1G5, 1B8, 2B3, 2C7, and 3EO as well as the Tuck tape (no batch number available) produced no background on the film detector, nor did one batch of 3M tape type 866. Tapes of the same type as the above (810) with batch numbers 1B2, 1K8, 2K5, and 2A7, as well as 3M tape with batch number AWS-2078 057 produced a very high number of background tracks on the detector and thus were useless for this purpose.

Neutron Sources. Two reactor neutron sources were used: the patient port at the MRR at Brookhaven National Laboratory and a facility at the Studsvik Science Research Laboratory, Studsvik, Sweden (20). Samples were exposed to between 10^12 and 10^14 thermal neutrons/cm^2.

Evaluation. After etching, the detectors were evaluated optoelectronically as described before (12) on a Quantimet 720 picture analyzer. An objective magnification of X4 of the microscope was used. A green filter was used to enhance contrast. A field size of 200 x 200 pixels was defined by the appropriate settings of the analyzer, framing out a field of 0.6 x 0.6 mm^2 on the object. The total number of pixels brighter than a chosen brightness level was recorded. Each individual track gave rise to about four bright pixels. The location of the area measured could easily be identified on the section, because most anatomical details also show up in differential boron uptake. When applicable, several areas of the same organ were evaluated and averaged in order to correct for possible freeze artifacts of the section.

RESULTS

Methodology. Fig. 1 shows typical whole-body cryosections of mice given injections of BSH and BSSB and their neutron capture radiograms, obtained by exposing the section to a flux of thermal neutrons in close contact with a cellulose nitrate track detector sensitive to α-particles.

Animals not given injections of boron compounds did not show any appreciable amounts of boron in their tissues (less than 0.1 ppm). Fig. 2 shows the number of bright pixels per field versus the amount of boron present in liver homogenates, exposed to 10^12 neutrons/cm^2 at the MRR and Studsvik, respectively. Several areas in each sample were counted and the SD determined. It can be seen that a very good correlation between the amount of boron and the number of bright pixels recorded is obtained and that the standards were quite uniform in their boron contents. The reproducibility between different slices of the same block and between different batches of standards was very good. By proper control of the etching conditions, a line with a slope of about unity was obtained.

The lower detection limit for the MRR was around 0.3 ppm boron (natural abundance) (0.06 ppm 10B). At this point, the number of pixels from background tracks from an empty standard was about one-half of the amount recorded in the presence of boron. Spurious tracks can originate from the neutron capture reaction in nitrogen [14N(n,p)14C] in the sample and in the detector, and from fast neutrons present in the beam. The Studsvik facility allows the quantification of boron down to 0.1 ppm (natural abundance, corresponding to 0.02 ppm 10B).

The standard curve obtained in this way was used for determining the amount of boron in the tissue samples on the same slice.

The high background from different batches of the same tape was disturbing. It might be speculated that some batches, but not all, contained boron (or less probably lithium or other nuclides undergoing n,α neutron capture reactions) from the manufacturing process of the glue, because no background tracks were found from the plastic itself.

For our purposes in this investigation, we have chosen very thick sections, which represent an infinitely thick layer for the particles generated in the neutron capture reaction. Thereby, the sensitivity should be maximal. Especially at the MRR, the sensitivity is limited by the amount of fast neutrons present in the beam. Thinner sections should diminish the sensitivity only slightly whenever there are no fast neutrons present. In this case, however, a uniform thickness of the section is of great importance for quantification, as observed by Abe et al. (17).

With such thick sections, the spatial resolution is in the order of 10-50 µm on the detector. It is estimated to about 0.1 mm for the correlation between detector and sample, which are separated after neutron exposure. However, because the measured field is much larger than this and because the histological quality of the section is usually poor, no attempts were made here to improve the spatial resolution. By choosing a field for measurements that is much larger than individual tracks, better statistics especially for small concentrations of boron are obtained.

Distribution of Compounds. An animal not given an injection of boron does not show great concentrations of boron (less than 0.1 ppm) in any part of its body, as judged by neutron capture radiography. Thus, all bright structures in neutron capture radiograms are due to the boron administered to the animal.

BSH is the compound used clinically for the treatment of brain tumors and has been demonstrated before (4) to show a relatively high uptake in the Harding-Passay melanoma used here as test model. This was essentially confirmed with QNCR (Fig. 3). The concentration in cerebrum was only 0.05 ppm 10B after 6 h and not measurable after longer times. Equally, the concentration in brain was very low (0.7 ppm) in any part of its body, as judged by neutron capture radiography. Thus, all bright structures in neutron capture radiograms are due to the boron administered to the animal.

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Uptake in the tumor stabilized after 12-24 h at a concentration of between 0.6 and 3 ppm in different parts of the same tumor, with very marked heterogeneity of uptake.

With BSSB (Fig. 4), uptake in the cerebrum of 0.3 ppm boron was found after 6 h, and the amount of boron dropped below the detection limit after 24 h. Also here, the uptake in the muscle was low. In contrast to BSH, uptake in the liver was...
QNCR IN TUMOR-SEEKING BORON-CONTAINING COMPOUNDS

Fig. 1. Top, whole-body sections (unstained) of mice sacrificed 12 h after i.p. injection of BSH (left) and BSSB (right) (35 µg ¹¹B/g body weight). Bottom, neutron capture radiograms of the same sections. Arrow, transplanted tumor; bar, 10 mm.

Fig. 2. Standard curve of number of bright pixels per 0.6- x 0.6-mm² field versus amount of boron present in the standards on a double-logarithmic scale. Saturation of the detector occurs at 40,000 pixels; the background level of the tissue is about 300 pixels. Bars, SD across one whole boron standard. Top curve, exposure at Studsvik; bottom curve, exposure at MRR; -, natural.

Fig. 3. Time course of boron distribution in different organs after i.p. injection of BSH (35 µg ¹¹B/g body weight). Boron concentration is plotted logarithmically. L, liver; B, blood; C, cerebrum; S, skin; M, muscle; H, heart muscle; U, lung; T, tumor. The detection limit in this experiment was 0.1 ppm ¹¹B.

DISCUSSION

The method for quantitative boron analysis in tissues and whole body presented in this paper has several advantages over the previously used methods of chemical analysis and prompt-γ analysis. The need for tedious dissection and sample prepa-

3 P. Micca and D. Slatkin, unpublished results.
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Fig. 4. Time course of boron distribution in different organs after i.p. injection of BSSB. Same conditions as in Fig. 3.

ratiom, as necessary in the two other methods, can be eliminated. No expensive equipment is needed, and the samples can be prepared in advance and sent by mail to the irradiation facility. (It was found advantageous to place the section onto the detector immediately before irradiation and separate the two immediately afterwards, because the glue of the tape will damage the detector upon prolonged contact.)

The use of tissue as a matrix for boron standards has advantages over other matrices. Texture, water contents, and shrinking during drying are very similar to the behavior of sample tissue. Furthermore, the number of tracks and radiation damage to the detector from 4N(n,p)4C reactions in the standard is the same as in the sample. It has been found previously that not only track-yielding particles but also exposure to other radiation influences the etching time and track yield of the detector.

Besides the ease of usage, this method gives information not available through either chemical or prompt-7 analysis, especially when small animals are used. It gives information about boron concentrations in small organs or parts of organs, and about inhomogeneities within one organ. The problems which arise with the other methods through precipitates in the peritoneal cavity are eliminated here. Precipitates have been found to lead to erroneously high boron values for internal organs when whole organs of dissected animals are analyzed. They therefore represent a major cause of error.

Compared with other methods of QNCR described in the literature (13, 14), the convenience of a standard preparation and its incorporation into the specimen as well as the ease of evaluation of the technique described herein reduces sample manipulations and speeds up the evaluation.

When the results obtained by QNCR for the biodistribution of BSH and BSSB are compared with the data obtained with prompt-7 spectroscopy (4), it is apparent that uptake in blood and especially cerebrum are consistently overestimated by the latter method. Thus, after 12 h, cerebrum values of BSH of 3-9 ppm obtained by prompt-7 measurements (4) should be compared with the values from QNCR, where boron is not detectable. Also the blood values are considerably overestimated by prompt-7 spectroscopy.

Due to the high sensitivity and the large dynamic range, QNCR allows the analysis of boron biodistribution even with compounds that are not 10B enriched. As mentioned above, such compounds would be cheaper to synthesize. With prompt-7 spectroscopy, analysis for boron of natural isotope abundance requires therapeutic amounts for detection. Even then, the evidently large errors at lower concentrations might lead to the elimination of promising compounds.

ACKNOWLEDGMENTS

We wish to thank Dr. P. W. Jungblut for help in the initial stage of this work.

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