Prompt Gamma Ray Spectrometry for In Vivo Measurement of Boron-10 Concentration in Rabbit Brain Tissue

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Abstract

Boron-10 concentrations in the brain of live rabbits were measured by prompt gamma ray spectrometry at intervals over a 24-hour period. Boron-10 concentrations in the blood and cerebrospinal fluid (CSF) were also measured. Animals were killed at each interval to obtain brain tissues to measure the boron-10 concentration in the brainstem, cerebral cortex, cerebellar cortex, and basal ganglia, as well as the whole brain. Boron-10 concentrations in the live brain did not differ significantly from those measured in whole brain tissue. Boron-10 concentrations in the blood were much higher than in the brain at each interval after injection. These boron-10 concentrations showed a similar pattern of initial rapid decrease, followed by a more gradual decrease. There was little boron-10 present in the CSF. The brainstem contained a significantly larger concentration of boron-10 than the other tissues. Prompt gamma ray spectrometry has the potential for direct measurement of boron-10 concentrations in the brain of patients undergoing boron neutron capture therapy.

Key words: brain tumor, boron neutron capture therapy, prompt gamma ray spectrometry, boron sulfhydryl

Introduction

Boron neutron capture therapy (BNCT) is based on the nuclear reaction between boron-10, which has a very large neutron capture cross section, and thermal neutrons. The nuclear reaction yields high energy (2.4 MeV) alpha particles with a short path length (10 nm), indicating that the particles will kill only cells containing boron-10. The energy of the thermal neutron is too small to endanger normal cells, as the elements in normal brain tissue (e.g. H, O, C, N, Na, K, Cl) capture only a minimal amount of thermal neutrons. Therefore, the efficacy of BNCT for curing brain tumors is mainly determined by two factors: the thermal neutron flux and the boron-10 concentration in the brain tumor.

Previous workers have developed a more concentrated and less contaminated thermal neutron beam, and a simultaneous monitoring system using acoustic pulse generation which allows easy continuous monitoring of the neutron flux in desirable areas. In contrast, boron-10 concentration can only be measured by quantitative biochemical analysis, which requires meticulous and time-consuming procedures. An accurate assessment of boron-10 concentration is impossible to obtain before neutron irradiation in clinical use. To achieve a better effect on brain tumors, the boron-10 concentration in the tumor tissue must be measured before deciding on the irradiation time.

The pharmacokinetics of boron compounds in brain tumor, normal brain tissue, and blood are also unknown. There is much basic information about various boron compounds, but little about the biological behavior in the brain tissue of living animals. Prompt gamma ray spectrometry allows
measurement of boron-10 concentration within a few minutes.\(^{(15)}\)

This experimental study used prompt gamma ray spectrometry to perform chronological in vivo measurements of boron-10 concentration in the brain tissue, blood, and cerebrospinal fluid (CSF) of the rabbit.

Materials and Methods

I. Experimental procedures

Adult New Zealand rabbits (n = 14) weighing 3–3.5 kg were used in this study. After induction of general anesthesia by intravenous injection of pentobarbital (30 mg/kg), a burr hole (7 mm in diameter) was made in the right parietal region of the skull using a micro drill. Following hemostasis of the blood from the dura mater and bone edge, the head was fixed in the measuring system (prompt gamma ray spectrometer). The beam guide (E-3) leading from the reactor at the Research Institute, Kyoto University was attached to the burr hole. Boron-10 as Na\(_2\)B\(_{12}\)H\(_{12}\)SH (BSH) (50 mg/kg) diluted in physiological saline was injected intravenously. The neutron beam was delivered through the burr hole (Fig. 1 upper). Gamma rays emitted from the brain were measured by a germanium (Li) detector\(^{(15)}\) to measure the boron-10 concentration. The boron-10 concentrations in the brain tissue of the living rabbits were measured every 10 minutes during the 1st hour after BSH injection, every 30 minutes during the 2nd and 3rd hour after injection, and every hour after the 3rd hour in 14 rabbits for a 3-hour period, in 10 rabbits for a 6-hour period, and in six rabbits for a 24-hour period. The rabbits were then sacrificed by KCl injection into the atrium. The brains were immediately removed and separated into the cerebral cortex, cerebellar cortex, basal ganglia, and brainstem. These four brain tissues were collected in pure Teflon tubes for boron-10 concentration measurement by prompt gamma ray spectrometry (Fig. 1 lower). The four brain tissues from each animal were then collected together and homogenized, and the boron-10 concentration of the homogenized whole brain measured. The data was compared with the value for the boron-10 concentration in the brain tissue of the living rabbit measured just before the sacrifice.

Blood samples (0.5–1.0 ml) from the veins and CSF from a suboccipital cisternal puncture were collected from the six rabbits monitored for 24 hours. The blood samples were drawn at the same times as the boron-10 concentrations were measured. The CSF samples were collected at six different times: 4, 6, 9, 15, 20, and 24 hours after BSH injection. These blood and CSF samples were also collected in pure Teflon tubes to measure the boron-10 concentration by prompt gamma ray spectrometry.

II. Theory and method of prompt gamma ray spectrometry\(^{(15)}\)

The concentrations of boron (B) and hydrogen (H) were assumed to be homogeneous in the tissue, so that prompt gamma rays emitted from H can be used for normalization. In the thermal neutron energy region, the cross sections of both B(n,\(\alpha\)\()^{7}\text{Li}^*\) and H(n,\(\gamma\))D have the 1/\(v\) characteristic, and the branching ratio of \(^7\text{Li}\)\(^*\) is constant as follows:

\[
^{10}\text{B} + n \rightarrow ^7\text{Li} + \alpha + 2.79 \text{ MeV (6.3%)},
\]

\[
\text{Li}^* + \alpha + 2.31 \text{ MeV (93.7%)},
\]

\[
\rightarrow \text{Li} + \gamma + 478 \text{ kV}
\]

The ratio of gamma rays emitted from the

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10B(n,γ)Li* reaction to those from the H(n,γ)D reaction (10B/H gamma ray ratio) is independent of thermal neutron irradiation conditions, as long as the distributions of 10B and H in the samples are homogeneous. Therefore, the 10B/H gamma ray ratio is proportional to the ratio of 10B to H atoms in the samples. A thermal neutron flux (5 mm in diameter, fluence rate $2 \times 10^6$ neutron·cm$^{-2}$·sec$^{-1}$ with a gamma ray contamination of about 1 mR/hr at an opening power of 5 MW) was generated from the reactor and passed through the neutron guide tube to irradiate the samples. 12 The prompt gamma rays emitted from the samples were counted by a germanium (Li) detector and normalized using the 10B/H gamma ray ratio.

Known samples with various boron-10 concentrations (0.01–100 ppm) were prepared in advance by diluting the JIS standard solution of 1000 ppm boron with distilled water. The boron-10 concentration in unknown samples could then be determined by comparison with known sample data as follows:

$$C_x = C_0R_xA_x/R_0A_0$$

where $C_x$: 10B concentration in unknown sample, $C_0$: 10B concentration in known sample, $R_x$: 10B/H gamma ray ratio of unknown sample, $R_0$: 10B/H gamma ray ratio of known sample, $A_x$: H concentration in unknown sample, $A_0$: H concentration in known sample (usually, $A_x = A_0$).

**Results**

The boron-10 concentration in the blood reached a peak of $457.0 \pm 21.0$ ppm (mean $\pm$ SD) at 10 minutes after the intravenous injection. The concentration then decreased rapidly to $50.7 \pm 6.2$ ppm at 60 minutes after injection. Subsequently, there was a gradual decrease to $19.4 \pm 1.5$, $8.9 \pm 0.9$, and $3.4 \pm 0.8$ ppm at 3, 6, and 24 hours, respectively (Fig. 2).

The boron-10 concentration in the live brain tissue reached a peak of $27.1 \pm 2.7$ ppm ($^{10}$B/H = 1.46 $\pm$ 0.135) at 10 minutes after injection, and then decreased to $8.3 \pm 1.4$ ppm ($0.48 \pm 0.076$) at 60 minutes. The concentration then gradually decreased to $3.9 \pm 0.6$ ppm ($0.21 \pm 0.032$), $2.4 \pm 0.2$ ppm ($0.13 \pm 0.011$), and $0.8 \pm 0.3$ ppm ($0.04 \pm 0.016$) at 3, 6, and 24 hours, respectively. The curve showed a pattern similar to that in the blood and no sign of re-elevation (Fig. 3).

The boron-10 concentration in the CSF was less than 0.5 ppm throughout the experiment (Table 1).

**Table 1** Concentration of boron-10 in the CSF after intravenous injection of BSH (50 mg/kg)

<table>
<thead>
<tr>
<th>Time after injection (hrs)</th>
<th>Boron-10 concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.43 $\pm$ 0.027</td>
</tr>
<tr>
<td>6</td>
<td>0.41 $\pm$ 0.014</td>
</tr>
<tr>
<td>9</td>
<td>0.12 $\pm$ 0.019</td>
</tr>
<tr>
<td>15</td>
<td>0.05 $\pm$ 0.023</td>
</tr>
<tr>
<td>20</td>
<td>0.03 $\pm$ 0.029</td>
</tr>
<tr>
<td>24</td>
<td>0.06 $\pm$ 0.011</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SD ($n = 6$).

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The boron-10 concentrations in the four brain tissues are shown in Fig. 4. The boron-10 concentrations in the cerebral cortex were $3.1 \pm 0.6$, $1.4 \pm$
The boron-10 concentrations in the whole brain were 3.7 ± 0.7, 1.8 ± 0.4, and 0.7 ± 0.1 ppm at 3, 6, and 24 hours after intravenous injection, respectively. There were no obvious differences between the values for the live brain and the whole brain. In contrast, the brainstem region had the highest boron-10 concentrations throughout the experiment (9.8 ± 1.1, 2.5 ± 0.4, and 1.7 ± 0.4 ppm at 3, 6, and 24 hrs, respectively).

Discussion

Clinical trials of BNCT were first attempted in the United States in the 1950s, but were discontinued in 1961 after several trials because of the discouraging clinical results. Important factors in this initial failure were the contamination of the neutron source and the inadequacy of the boron compounds. Hatanaka et al. found a new boron-10 compound: sodium mercaptoundecahydrododecaborate (BSH) and demonstrated the selectivity of accumulation in tumor tissue. They revived BNCT in 1968 at the Hitachi Training Reactor by modifying the procedure of Sweet and Javid to treat 53 patients with brain tumors between 1968 and 1982. Nine of the 53 patients survived for longer than 10 years. These long-term survivors showed no evidence of tumor recurrence, and most could maintain a good quality of life. These excellent results encouraged many further efforts to improve the results of BNCT, in the fields of physics, chemistry, and medicine.

Effective BNCT of brain tumors requires greater accumulation of boron-10 in the tumor tissue than in the normal brain tissue. Sweet et al. collected biopsy samples at 38–145 hours after arterial injection of boron-10 in clinical cases of brain tumors (glioblastoma) to conduct biochemical analyses. The boron-10 tumor/normal brain ratios were 1.6–7.8. Stragliotto and Fankhauser measured the boron-10 concentrations in biopsy samples from 29 patients with intracranial tumors at 18 and 24 hours after intravenous injection. All boron-10 tumor/normal brain ratios were above 2.0. These data suggest the potential efficacy of BNCT, but the pharmacokinetics of BSH are still unclear.

There are remarkable individual variations between patients in the changes in boron-10 concentration in brain tissue, tumor tissue, and blood. Sweet et al. collected biopsy samples at 38–145 hours after arterial injection of boron-10 in clinical cases of brain tumors (glioblastoma) to conduct biochemical analyses. The boron-10 tumor/normal brain ratios were 1.6–7.8. Stragliotto and Fankhauser measured the boron-10 concentrations in biopsy samples from 29 patients with intracranial tumors at 18 and 24 hours after intravenous injection. All boron-10 tumor/normal brain ratios were above 2.0. These data suggest the potential efficacy of BNCT, but the pharmacokinetics of BSH are still unclear.

There are remarkable individual variations between patients in the changes in boron-10 concentration in brain tissue, tumor tissue, and blood. Sweet and Javid measured the boron-10 concentrations in the normal brain tissue and blood of rats by biochemical analysis, finding that the boron-10 concentrations in the blood and normal brain tissue reincreased roughly 12 hours after injection. Our chronological in vivo measurements, however, found that the boron-10 concentration decreased gradually from the peak at 10 minutes and no phenomenon of reelevation was observed. Our results agree with those of Abe et al. who used autoradiography to measure the boron-10 concentration in normal rat brain tissue, finding 3.1 and 2.0 ppm in normal brain tissue at 3 and 13 hours after injection, respectively.

The boron concentration has been measured clinically using positron emission tomography or boron magnetic resonance imaging. However, the accuracy is still not adequate for clinical trials. Prompt gamma ray spectrometry has been used in clinical trials, but requires debulking of the tumor tissue to obtain specimens. The chronological measurement of the boron concentration in normal brain tissue or brain tumor in vivo is still impossible, due to the following problems. The blood in the surrounding structures (dura, cranial bone marrow, scalp, blood vessels, etc.) may also be irradiated with the neutron beam, thus contributing to a higher boron-10 concentration measurement. Loss of blood occurring when the tumor tissue is debulked or animals are sacrificed may lower the boron-10 con-
centration. Individual variations can cause differing measurement values.

We conducted our initial experiments using rats. However, the head size did not allow a large enough burr hole for the 5 mm thermal neutron flux. In the investigation, the boron-10 concentrations recorded in live brain tissue were much higher, possibly because the higher blood boron-10 concentration in the surrounding bone marrow had affected the measurement. Our present measurements of the boron-10 concentration in live brain tissue by prompt gamma ray spectrometry recorded the boron-10 concentrations in the whole brain, not only in the cortex. There was no obvious difference between the boron-10 concentration of the living brain and the sacrificed whole brain, indicating that prompt gamma ray spectrometry can be used to measure chronologically the boron-10 concentration in the brain tumor and brain tissue during clinical BNCT.

The blood volume in the brain is estimated to be about 5-8% of the total brain tissue. In our study, the boron-10 concentration in the living brain tissue was about 6% of the boron-10 concentration in the blood at 10 minutes after injection. Thus, at 10 minutes after injection, little boron-10 had moved from the blood to the brain tissue. At 60 minutes, the proportion had increased to about 16%, and then to about 20%, 27%, and 24% at 3, 6, and 24 hours after injection, respectively. The boron-10 concentration in the CSF did not increase during the experiment. This suggests a shift of boron-10 from the blood to the normal brain other than the CSF. We assumed that approximately 10-20% of the boron-10 in the blood penetrated into the normal brain tissue. The study of separate brain tissues showed the boron-10 concentration in the brainstem was the highest, indicating that the movement of boron-10 from blood to normal brain tissue mostly occurred in the brainstem region.

Our group (at the National Kagawa Children’s Hospital and The University of Tokushima) has performed BNCT in more than 40 patients with malignant brain tumors. Clinically, the boron-10 concentration in the patient’s blood and the removed sample of brain tumor could be easily and quickly measured by placing the samples into pure Teflon tubes for prompt gamma ray spectrometry. The effective irradiation dosages (ca. 15 physical gray of boron neutron-\(\alpha\) wave reaction) could be determined immediately to kill the tumor cells efficiently and to protect the brain tissue and endothelium of the blood vessels. However, chronological measurement of the boron concentration in the brain tumor and in the normal brain tissue around the tumor by a noninvasive method would allow much more accurate radiation planning. We believe that the development of prompt gamma ray spectrometry methods will improve the curative effects of BNCT on malignant brain tumors and help prevent damage to the normal brain.

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