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Application of neutron capture autoradiography to Boron Delivery seeking techniques for selective accumulation of boron compounds to tumor with intra-arterial administration of boron entrapped water-in-oil-in-water emulsion

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ABSTRACT

It is necessary to accumulate the ^{10}B atoms selectively to the tumor cells for effective Boron Neutron Capture Therapy (BNCT). In order to achieve an accurate measurement of ^{10}B accumulations in the biological samples, we employed a technique of neutron capture autoradiography (NCAR) of sliced samples of tumor tissues using CR-39 plastic track detectors. The CR-39 track detectors attached with the biological samples were exposed to thermal neutrons in the thermal column of the JRR3 of Japan Atomic Energy Agency (JAEA). We obtained quantitative NCAR images of the samples for VX-2 tumor in rabbit liver after injection of ^{10}BSH entrapped water-in-oil-in-water (WOW) emulsion by intra-arterial injection via proper hepatic artery. The ^{10}B accumulations and distributions in VX-2 tumor and normal liver of rabbit were investigated by means of alpha-track density measurements. In this study, we showed the selective accumulation of ^{10}B atoms in the VX-2 tumor by intra-arterial injection of ^{10}B entrapped WOW emulsion until 3 days after injection by using digitized NCAR images (i.e. alpha-track mapping).

1. Introduction

A malignant tumor should be removed from a body or be under an all-out attack with some means. One of the effective methods is Boron Neutron Capture Therapy (BNCT) for tumors. The cytotoxic effect to tumors is caused by high linear energy transfer (LET) particles produced from a nuclear reaction between ^{10}B and thermal neutrons in the tissue. These nuclear interactions are mainly as follows:

$$^{10}B + {}^{1}n \rightarrow {}^{7}Li + {}^{4}He\ (\alpha) + 2.31\,MeV\ (93.7\%)$$
$$\rightarrow {}^{7}Li + {}^{4}He\ (\alpha) + 2.79\,MeV\ (6.3\%).$$

These high LET particles (α and ^{7}Li) destroy cells within about 10 μm path length from the site of capture reaction. Therefore, ideally it is possible to kill tumor cells without affecting adjacent normal cells, if sufficient ^{10}B atoms can be selectively accumulated in tumor cells. Therefore, one of the most important concerns in BNCT is the accurate measurement of ^{10}B concentrations and

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distributions in biological samples, in order to evaluate the usefulness of various ^{10}B-delivery compounds.

In the previous study, we employed a technique of neutron capture autoradiography (NCAR) using CR-39 plastic nuclear track detectors and determined the ^{10}B-concentrations and distributions in whole body sections of mice [1–5]. CR-39 plastic track detectors have high potential ability for imaging analysis NCAR because of its easy handling and enough sensitivity to α- and Li-particles from ^{10}B(n, α)^{7}Li reaction. These studies have been done by α track counting in NCAR images using the semi-automated optical microscope equipped with CCD camera. Under the optical microscope observation, typical image sizes are limited to several hundred micrometers square of the detector surface. Thus, it is excessively time consuming to scan a NCAR image.

Recently, one of the authors (N. Yasuda) developed a high-speed imaging microscope and new software for nuclear track detector analysis [6]. This analyzing system, named HSP-1000, is able to scan and analyze particle tracks (etch pits) with image acquisition speed of 50–100 times faster than conventional microscope analyzing systems [6]. In this study, the ^{10}B accumulations and distributions in VX-2 tumor and normal liver of rabbit were estimated by means of alpha-track density measurements using HSP-1000. An example that the injected ^{10}B atoms were selectively accumulated in the VX-2 tumor is shown using a digitized NCAR image constructed by alpha-track mapping. The digitized image reconstruction method is also described briefly.

2. Materials and experimental methods

2.1. Preparation of Boron entrapped WOW emulsion

Three hundred milligrams of ^{10}BSH ($Na_2{}^{10}B_{12}H_{11}SH$) was dissolved in 5 ml of 5% glucose solution, filtered through a controlled pore glass membrane emulsified into 5 ml of Iodinated poppy seed oil (IPSO) containing surfactant forming the water-in-oil emulsion (WO). The WO emulsion was emulsified again with an aqueous phase containing 5 ml of saline and surfactant. The ^{10}BSH entrapped water-in-oil-in-water (WOW) emulsion was prepared with this double emulsifying technique [7–9]. The concentration of ^{10}B entrapped in WOW vesicles was determined by ICP-Mass spectroscopy at Jyuntendo University.

2.2. Preparation of the sample for NCAR experiment

The VX-2 (Shope-virus derived Squamous Cell Carcinoma cell line) cells were inoculated into the foot pad of a rabbit and the tumors of VX-2 were allowed to form for 1 week. The nodules of VX-2 tumor were inoculated into the left hepatic lobe of the liver and the hepatic tumor models were constructed for 2 weeks. In order to examine the accumulations of the ^{10}B atoms, the rabbits were sacrificed 3 days after the intra-arterial injection of ^{10}B entrapped WOW emulsion, and then the hepatic samples were frozen at −60 °C. Subsequently, the frozen hepatic samples were cut sagittally into 40-μm-thick sections mounted on a thin 3M Scotch tape, and freeze-dried at −20 °C for 2 weeks and air-dried for 1 more week.

2.3. Neutron irradiation and Etching procedure

The CR-39 plastic (also known as PADC for polyallyl-diglycol carbonate) nuclear track detectors are sensitive to charged particles of LET greater than 5 keV/μm. Eight samples of sliced hepatic sections were put in close contact with the CR-39 (HARZLAS TD-1; Fukuvi Chemical Industry, Japan) plastic plate ($93 \times 93 \times 0.95$ mm thick) using thin adhesive tape (Scotch Transparent Tapes acetate film, type 810-3-18; 3M, USA). The CR-39 nuclear track detectors attached with the sliced hepatic sections were exposed to thermal neutrons in the thermal column of the JRR3 of Japan Atomic Energy Agency (JAEA). The thermal neutron fluences varied according to the objectives of the experiments and were 2×10^{12} neutrons/cm^2 for the emergence of visible image, and 2×10^{10} neutrons/cm^2 for the track measurement. After the irradiation, the CR-39 detector plates were etched in a 7 N NaOH solution at 70 °C for 2 h to reveal tracks for NCAR imaging.

2.4. Scanning procedure

A high-speed image acquisition microscope (HSP-1000) that uses a line sensor camera in place of a traditional CCD camera has been developed [6]. The HSP-1000 is a new microscope system for capturing large images (>1 cm^2) in relatively short periods of time (<1 min). Continuous, automatic focusing of the microscope is achieved by means of an optical pick-up system that provides fast feedback for control of distance between the objective and the image surface. Using transmitted light illumination, the microscope is able to digitize a 1 cm^2 area at 0.35 μm/pixel resolution in ~20 s. Due to the continuous stage motion and continuous focusing, the HSP-1000 has a capability of image acquisition speeds that are 50–100 times faster than conventional CCD-based microscope systems. The HSP-1000 is capable of accurate measurements of two-dimensional track positions and the image of individual tracks recorded on a large area of CR-39 plate. The system is stored with scanning image data on a hard disk drive. The greyscale image is converted to a binary image based on a user-set greyscale threshold. The image is searched for features that possess the signature pattern of nuclear track etch pits. An ellipse is then fit to the opening of each etch pit with application software for the HSP-1000 [6].

The CR-39 plates irradiated by 2×10^{12} neutrons/cm^2 were used only for the confirmation of the accumulation of ^{10}B in the liver tissues, because a large number of α tracks overlapped each other and formed high contrast NCAR images corresponding to the areas of high ^{10}B concentrations. It is easy to recognize ^{10}B accumulations in the sample by naked eye observation, but CR-39 plates irradiated by 2×10^{12} neutrons/cm^2 is useless for track analyses due to the overlapping of tracks.

The visible NCAR images do not appear on the CR-39 plates irradiated by 2×10^{10} neutrons/cm^2 due to less track densities. Therefore, referring to the NCAR images on CR-39 plates irradiated by 2×10^{12} neutrons/cm^2, the tracks on CR-39 plates irradiated by 2×10^{10} neutrons/cm^2 were automatically measured using HSP-1000. The track area of the opening of each track as well as its position was analyzed. In this paper, we discuss and demonstrate the results only for the sample extracted from the rabbit 3 days after the injection of ^{10}B-WOW emulsion.

3. Results and discussion

An example of CR-39 image scanned with the HSP-1000 is shown in Fig. 1. The larger ellipse track images originated from α particles and the smaller ones from proton tracks. It is considered that the contribution of Li tracks is not so large because of its shorter range. The opening area sizes of each track (etch pit) were obtained using the ellipse fitting application software for the HSP-1000.

Fig. 2 shows a two-dimensional scatter plot (i.e. track mapping) of the coordinate of each track observed for whole sliced liver sections of the sample. In other words, this kind of