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Boron Neutron Capture Radiography—Perspectives in Melanoma Therapy

Bengt S. Larsson

Department of Toxicology, Uppsala University, Uppsala Biomedical Center, Box 594, S-751 24 Uppsala, Sweden

Over the past decades, much interest has been focused on the possibility to develop drugs with selective affinity for receptors or other components of tumour cells as a basis for diagnosis and therapy of malignant neoplasms. By using such compounds as carriers of stable nuclides, e.g. ^{10}B, that can later be activated by neutron capture, an improvement in therapeutic efficacy and reduction of side effects may be combined. A distinct advantage of this approach is that metastases would be targeted automatically. The present paper is briefly dealing with boron neutron capture as a tool for studying the distribution of certain boron-containing substances in experimental animals and, in the prolongation, for possible boron neutron capture therapy of malignant melanomas.

Naturally occurring boron consists of 19.6 % ^{10}B which has a large cross section for thermal neutrons (3840 barns). After the capture of a slow (thermal) neutron, the boron nucleus undergoes instantaneous nuclear fission into an alpha particle and a lithium ion according to the following scheme:

$$^{10}B + {}^{1}n \text{ ——— } \alpha\,(1.78) + {}^{7}Li\,(1.01) + \gamma\,(0.48)$$

The kinetic energies of the particles in MeV are given in brackets (14). The emitted particles exert radiochemical action along short (< 9 microns) tracks, i.e. within the diameter of single cells. Due to the high-linear energy transfer of this radiation, large biological effects might be restricted to the ^{10}B-containing tissues without cell cycle and dose-rate effects.

To obtain effective therapy by boron neutron capture, it is essential to prepare stable, nontoxic, substances rich in ^{10}B and with specific affinity for tumour cells. A limiting factor regarding maximal neutron dose is the neutron capture reactions in nitrogen $/^{14}N\,(n, p)\,{}^{14}C/$ and hydrogen $/^{1}H\,(n, \gamma)\,{}^{2}H/$ of the normal tissue. Other important parameters are the ^{10}B ratio (tumour / normal tissue), ^{10}B concentration, and the target depth. For example, the necessary ^{10}B concentration is of the order 28-36 μg/g tumour, if the ^{10}B ratio is 10 and the depth is 4 cm (8).

The initial clinical trials with boron neutron capture therapy gave poor results, mainly due to the lack of suitable carriers of ^{10}B in combination with the rapid attenuation of thermal neutrons in tissue (7, 8). During the last years, however, different promising substances have been identified as possible vehicles for the delivery of ^{10}B to tumours, including antibodies, amino acids, porphyrins, nucleosides, steroides, and phenothiazine derivatives (for review, see ref. 7, 8). Improvement of the quality of thermal and epithermal neutron beams, free from fast neutrons and gamma contamination, has also currently been obtained, making neutron capture therapy more attractive.

Before clinical application, the biological fate of boronated compounds must be thoroughly investigated in experimental animals. Since no radioisotopes of boron are available for autoradiography, a new technique, named boron neutron capture radiography (BNCR), was developed, based on the ^{10}B (n, α) ^{7}Li reaction (14, 19, 20). The first experiments with BNCR for the localization of boron in histological sections were performed with photographic emulsions as detectors (6, 9, 13, 20). A drawback of this technique was the heavy background that appeared, mainly due to the nitrogen of the gelatine that builds up the emulsion; the nitrogen undergoes the ^{14}N (n, p) ^{14}C reaction during neutron exposure. In 1967, Hughes and Rogers (12) developed a technique for revealing the microdistribution of boron in surfaces of solids by BNCR using certain plastics as solid state detectors of the radiation. These plastics (10, 11) do not record beta or gamma radiation and contain no nitrogen. The invisible microtracks in the plastics, caused by the alpha particles and the lithium ions, were "developed" by etching in hot aqueous KOH, allowing the tracks to grow to sufficient size to be recorded under microscope.

Matsuoka et al. (19) were using cellulose nitrate plastics as detectors in distribution studies of boronated substances in mice, bearing transplanted tumours. They were preparing whole-body cryosections from the mice according to Ullberg (27, 28) and after freeze-drying, the sections were apposed to the plastic films and exposed to thermal neutrons. The films were etched by immersion into hot 6N NaOH solution. Börje Larsson and his group have further developed this technique (2, 14) and their results show that it may be possible to obtain useful experimental information from sections with a ^{10}B content down to about 0.1 µg per g tissue (14). Their studies were made on freeze-dried 5-20 µm thick sagittal sections from rats, injected with boronated macromolecules. The detectors were 6 or 12 µm thick cellulose nitrate films (Kodak-Pathé LR 115, types I and II) and during the neutron irradiation, the films and sections were pressed tightly together in an evacuated bag of thin polyethylene (14). The etching was performed with 2.25-6.25 N NaOH at 60 ^{o}C for varying periods (15-40 min). After etching, the films were washed for 30 min in water.

In cooperation with Börje Larsson and his colleagues we have started experiments on the accumulation of boron-labelled substances in melanotic melanomas transplanted

to mice. The BNCR technique used is similar to that described above (2, 14). The neutron irradiation has been performed in Studsvik, Sweden, with an equipment consisting of a large moderator tank, containing heavy water, in close contact with the R2-O reactor (2). These facilities permit whole-body sections to be exposed in their entirety by a flux of pure thermal neutrons.

Our experiments are based on previous findings that various substances are accumulated in melanin-containing tissues, including melanotic melanomas. The main part of these compounds, e.g. polycyclic amines such as chlorpromazine and chloroquine, are bound to the melanin by electrostatic interactions (15, 18, 25) – cf. the article by N.G. Lindquist in this journal. Attempts to scan melanomas by the use of radiolabelled substances with melanin affinity have been reported (1), but a drawback of this approach is the heavy binding to the melanin-bearing normal tissues. We have also found that thioamides, e.g. thiouracil, are accepted as false precursors of melanin during synthesis and strongly accumulate in murine melanomas (3, 4, 5, 16, 24). A characteristic feature of these substances is that they lack adsorption to already formed melanin (5) which means that they are exclusively accumulated in growing melanin. After radioiodination, thiouracil is still selectively incorporated as a false precursor into melanin (17), and it might therefore be used in the clinical diagnosis of malignant melanotic melanomas – clinical experiments with ^{131}I-thiouracil are in progress.

Therapy of malignant melanomas by the use of radiolabelled thioamides is scarcely practicable, due to the high radio doses that would be needed. As an alternative, thiouracil may be used as a carrier of ^{10}B for neutron capture therapy. The polycyclic amines, that are bound to preformed melanin, are also interesting as possible ^{10}B carriers. Mishima (21-23) has performed promising therapeutic studies with boronated analogs of melanoma-seeking compounds on experimental animals. An advantage of this technique is that patients could be administered high doses for long periods without any serious exposure to radioactivity.

We have developed a method for the synthesis of boron-containing melanoma seekers, both a thiouracil derivative (26) and some polycyclic amines, e.g. chloroquine and chlorpromazine analogs. The boron moiety has consisted of decaborane. Further preparation of suitable compounds is in progress – problems concerning solubility and chemical stability are still partly unsolved. The fate of these boronated substances in mice with transplanted Harding-Passey melanomas or B-16 melanomas has been studied by BNCR (cf. above). Preliminary results indicate that the boronated thiouracil derivative is accumulated in melanomas. Complementary studies, with conventional chemical analysis of the boron content of melanoma fractions, have shown that, on a weight basis, the melanin fraction contained 20 times more boron than the corresponding tumour tissue, indicating that the boronated thiouracil derivative was incorporated

into the melanin (26). The boron-containing chloroquine analog is also accumulated in the experimental melanomas (Fig. 1), apparently due to binding to the preformed melanin of the tumours.

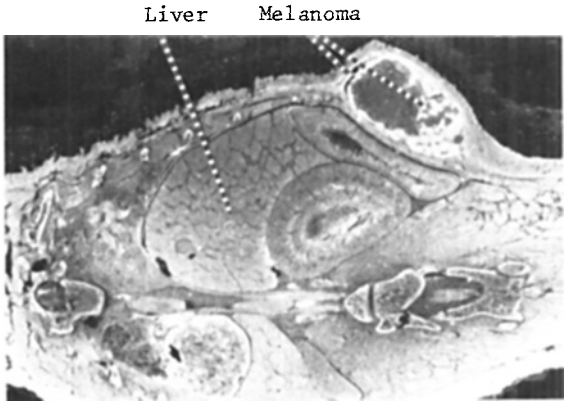

Fig. 1. Distribution of boronated chloroquine in a mouse with transplanted Harding-Passey melanoma, revealed by neutron capture radiography. The mouse was injected i.p. 6 times during 6 days with a total boron dose of 1.9 mg. Note the distinct uptake in pigmented parts of the tumour – the central region is necrotic.

CONCLUSIONS

The selective uptake of boronated substances in tumours is a necessary basis for neutron capture therapy. The biological fate of such compounds in experimental animals, transplanted with tumours, may be readily investigated by neutron capture radiography. In radiographic studies on boronated analogs of substances that are bound to preformed melanin (e.g. chloroquine) or incorporated into melanin during synthesis (thiouracil derivative), we have found that they are localized in murine melanotic melanomas, obviously due to interaction with the melanin. So far, the results are preliminary. Further studies are needed to evaluate the clinical potential of these compounds for melanoma therapy.

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Address for reprints:

Bengt Larsson

Uppsala University

Department of Toxicology

Uppsala Biomedical Center

Box 594

S-751 24 Uppsala, Sweden