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Monitoring of the Boron Neutron-Capture Reaction by a Simple Animal Model

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Summary

Sodium borocaptate (BSH, $Na_2B_{12}H_{11}SH$), a slow neutron-capture compound, was injected into the left forebrain ventricle of 1-week-old rats (150 μg BSH/3 μl phosphate buffered saline). After 90 min, the animals were irradiated by epithermal neutrons (LVR-15 nuclear reactor in Řež near Prague, flux density 8.8 x 10^7 neutrons cm^{-2} s^{-1}, 8 MW reactor power, 8.2 cGy/min) for 5, 10 or 20 min. The brains were examined histologically 8 h after irradiation. In animals irradiated for 5 to 10 min (41 and 82 cGy-Eq, respectively) lethal damage of cells was found in the external granular layer of the cerebellum and the subependymal layer of the forebrain. Irradiation for 20 min (164 cGy-Eq) caused more extensive destruction of cell populations in these regions and, in addition, dead cells appeared also in the more differentiated postmitotic compartments, namely the deeper layers of the cerebellum, layers II/III of the cerebral cortex and corpus callosum. In the forebrain periventricular layer, the extent of cell damage was declining towards the olfactory bulbs. In intact animals, as well as in those injected only with the 150 μl phosphate buffered saline, the radiation damage was low and limited only to the most sensitive dividing populations of the cerebellum and the forebrain. The study demonstrates a differentiation-dependent damage of the rat brain cells by alpha particles and presents a simple model for evaluation of the biological effectiveness of slow neutron beams constructed for neutron-capture therapy of tumors.

Key words

Brain tumors – Boron-neutron-capture therapy – Sodium borocaptate – Cell death – Radiation damage – Epithermal neutrons

Introduction

Boron neutron-capture reaction occurs after interaction of slow thermal neutrons with atoms of stable boron-10 isotope. It leads to release of alpha particles with energy 8 orders higher than that of colliding neutrons (Kliegel 1980, Barth *et al.* 1990, Burian *et al.* 1997). In addition, the alpha particles have high linear energy transfer and their energy is absorbed practically within the cell in which the reaction has been initiated. Unlike other types of radiation, biological effects of alpha particles are less oxygen-dependent, i.e. they destroy also the cells in hypoxic parts of tumors. The lesions of DNA are more difficult to be repaired. The boron neutron-capture therapy (BNCT) represents, therefore, a new way for a more efficient and selective destruction of tumor cells, namely those dispersed throughout the healthy tissues and out of the possibility of a surgical sanation. Compared to standard radiotherapy, destruction of tumor cells by neutron-capture reaction represents lower radiation load for the healthy tissue (Hatanaka *et al.* 1986). Successful application of BNCT presumes selective enrichment of tumor cells by boron-10.

Results were preliminary presented at "CNS – Advance in Research of Normal and Neoplastic Cells" which was held in Brno (April 25, 1996) as the satellite minisymposium of the 42nd International Congress of the European Tissue Culture Society (Mareš et al. 1996).

Another limit of this approach is low penetration of thermal neutrons into the larger or deeper located tumors. This problem can be solved by application of more energetic beams of epithermal neutrons which are slowed-down, and/or thermalized, in the irradiated tissue. However, the beams of epithermal neutrons are unavoidably contaminated by some parasitic radiation, namely the fast neutrons and gamma rays formed during the process of thermalization of epithermal neutrons in the tissue. This represents a potential ionization danger for normal cells without therapeutic levels of boron-10. Construction of the therapeutic beams of thermal or epithermal neutrons is a High-Tech task for most advanced world nuclear centers. Here, we report biological effects of an epithermal neutron beam set-up at the LVR-15 nuclear reactor in Řež near Prague (Marek and Burian 1994, Burian *et al.* 1996, 1997) for a forthcoming BNCT of brain tumors. As a biological testing object, we used brain of early postnatal rats which provide a wide scale of cells with different degree of maturation and radiosensitivity (Mareš and Brückner 1978, Brückner *et al.* 1980, Jacobson 1991). Sodium borocaptate (BSH), a compound designed for BNCT of brain tumors, was applied as a neutron-capture agent (Hatanaka *et al.* 1986, Stragliotto and Fankhauser 1995, Horn *et al.* 1996 and others).

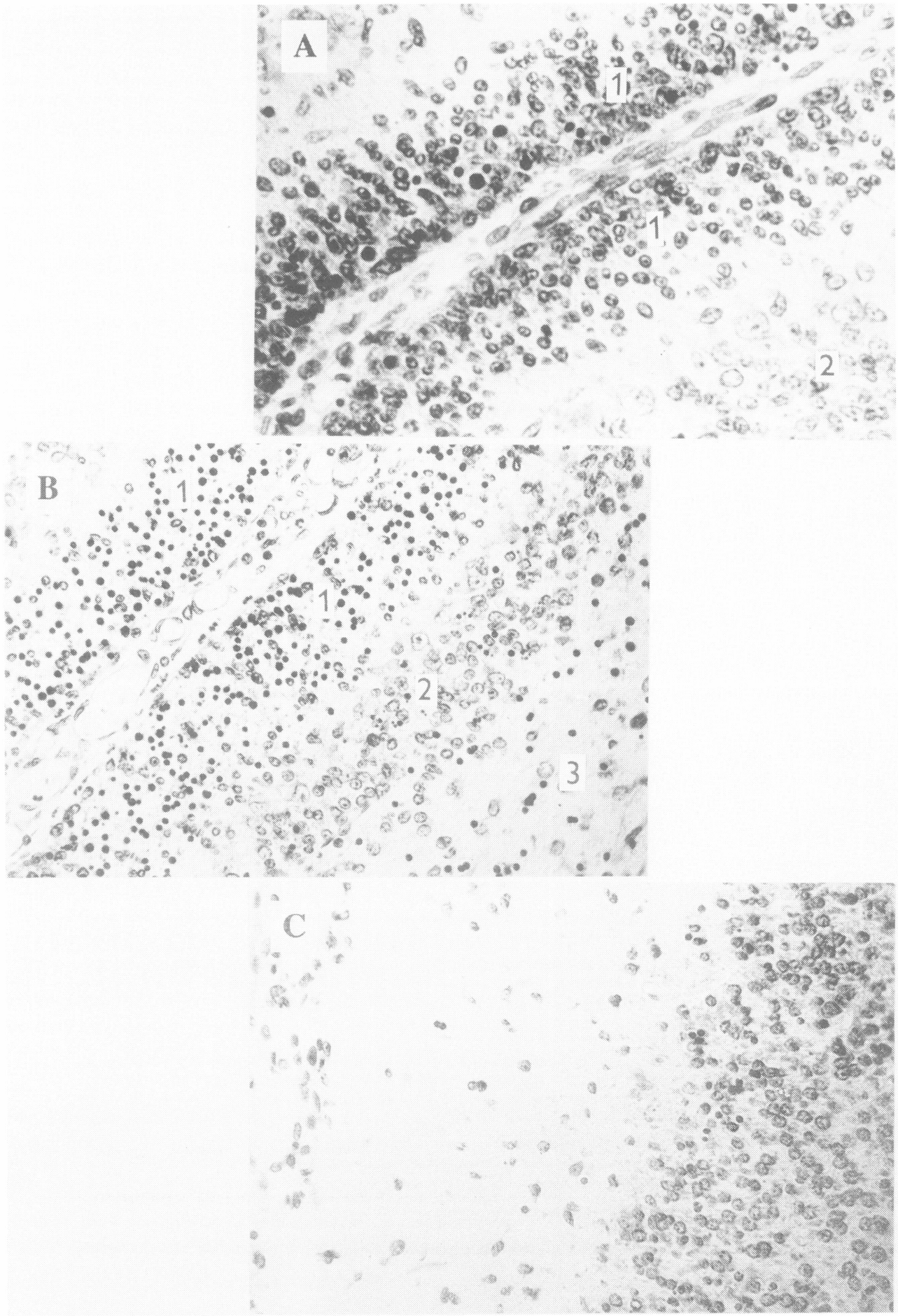
Material and Methods

One-week-old Wistar rats of both sexes (Inst. Physiol., Acad. Sci., Prague) were injected into the left fronto-parietal region of the brain either with 150 μl of phosphate buffered saline (pH 7.2) or mercapto-undecahydro-dodecaborate (BSH, $Na_2B_{12}H_{11}SH$, sodium borocaptate, 50 μg/ul phosphate buffered saline, natural content of boron-10, Plešek *et al.* 1985) using a 10 μl Hamilton microsyringe with a firm transversal bar 3 mm from the tip of the needle (Mareš *et al.* 1984). The calculated amount of boron-10 was 29.56 μg/g of the brain tissue. Ninety min after BSH injection the animals were irradiated by epithermal neutron beam (LVR – 15 reactor in Řež) thermalized by a 2.5 cm block of polyethylene . The thermal neutron flux density was 8.8 x 10^7 n cm^{-2} s^{-1} (8 MW reactor power) and the calculated physical dose was 8.2 cGy/min. This includes also unavoidable contamination of the beam by fast neutrons and gamma rays (2.03 cGy/min and 0.51 cGy/min, respectively). The time of irradiation was 5, 10 and 20 min, i.e. equivalents of 41, 82 and 164 cGy/min were applied. The intact and saline-injected control animals were irradiated in parallel under the same irradiation conditions. Animals were sacrificed by fast decapitation 8 h after being irradiated. The brains were examined in hematoxylin or toluidine blue stained sagittal sections prepared from the brains fixed in Carnoy solution (6:3:1), embedded into Paraplast-Plus (Polysciences) and cut sagitally from the right hemisphere. The radiation effect was expressed either as a total number of dead cells per an arbitrary area delineated in the ocular grid or as percentage related to all cells in the concerned region. In total we used 35 animals, with 3 to 4 per one experimental group.

Results

Non-irradiated animals injected intracranially with saline or BSH did not show changes in their behaviour except a few second lasting decrease in tonus of the trunk muscles at the end of BSH application. The brains of intact- and saline-injected animals appeared macroscopically normal except a fine dot hemorrhage on the surface of the fronto-temporal cerebral cortex of the injected left hemisphere. The brains of animals irradiated 90 min after injection of BSH were macroscopically swollen. Microscopic examination of animals irradiated in presence of BSH for 5 min revealed cells undergoing cell death, characterized by dense condensation of chromatin and apoptotic-like bodies in the external granular layer of the cerebellum and the subependymal layer of the forebrain (Fig. 1a,b). A small number of damaged cells appeared also in the hippocampus, mainly the hillus and the subgranular layer of the dentate gyrus. After irradiation of BSH-injected animals for 10 and 20 min, damaged cells appeared also in the more differentiated internal granular layer and medulla of the cerebellum, as well as layers II/III of the cerebral cortex (Fig. 1c). Damaged cells appeared in a small number also in the corpus callosum of animals irradiated in presence of BSH for 20 min. It is to be pointed out that a certain damage occurred also in the brain of animals irradiated without BSH. The number of affected cells was, however, much lower than in the animals irradiated in presence of BSH (Fig. 2).

Fig. 1. *Histology of the immature rat brain irradiated by epithermal neutrons in absence (A) and presence of BSH (B, C). A and B: cerebellar cortex with the external granular layer covering 2 adjacent folias (1), internal granular layer (2) and the medulla (3) C: the cerebral cortex, layers I and II/III from the left to right. The small black dots correspond to damaged cells undergoing apoptosis (B,C). The larger dense and less regular structures in a control animal (A) are mitosis. Irradiation time 20 min, hematoxylin staining, magnification 40x.*


A
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C

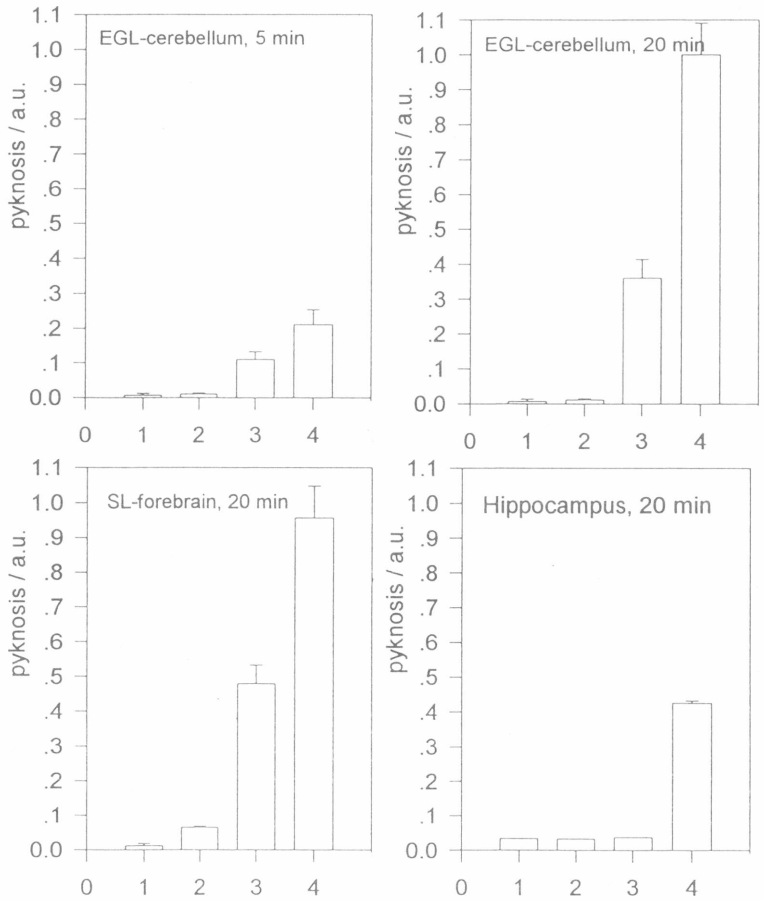

Fig. 2. *The incidence of pyknotic cells in the external granular layer (EGL) of the cerebellum, the subependymal layer (SL) of the forebrain and hippocampus of the immature rat brain. Irradiation time 5 and 20 min. Description of columns: 1 = intact or saline injected animals. 2 = non-irradiated animals injected with BSH. 3 = animals irradiated without BSH 4 = animals irradiated 90 min after i.c. injection of BSH. Statistical significance of the differences between the animals irradiated in absence and presence of BSH (columns 3 vs. 4) ranged from $p<0.05$ (EGL of the cerebellum, 5 min) to $p<0.01$ (20 min irradiation, all regions studied).*

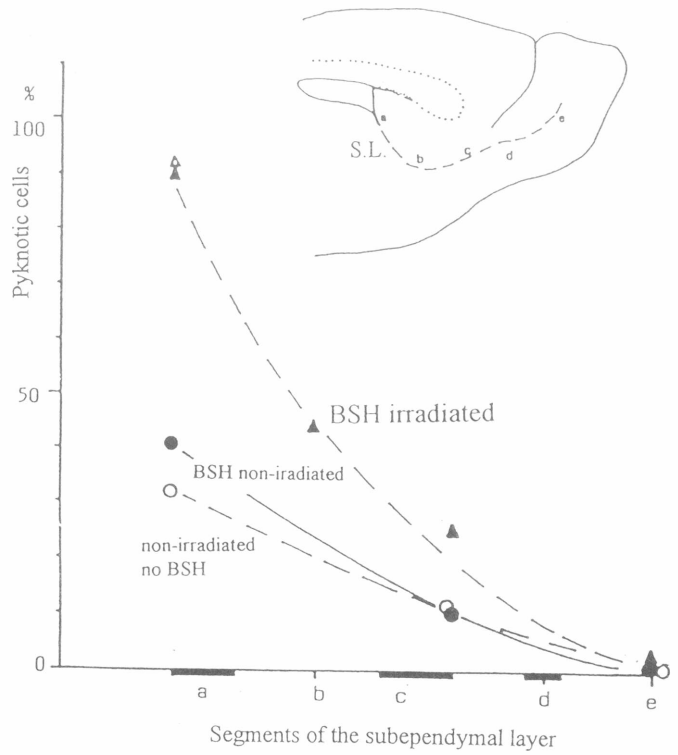

Fig. 3. *The incidence of damaged cells in different parts of the periventricular subependymal layer (SL) of the immature rat forebrain. Irradiation time 20 min. The arbitrarily selected 5 segments are labelled by letters a, b, c, d, e both in the anatomical scheme of the forebrain and under abscissa of the plot. For better illustration, the measured values were fitted by exponential curves. The values represent means of 2 to 3 animals.*

Furthermore, damaged cells in animals irradiated without BSH appeared only in the most immature external granular layer of the cerebellum and the subependymal layer of the forebrain while in the regions with postmitotic cells (e.g. internal granular and medullary layers of the cerebellum, cerebral cortex) no signs of destruction were found. In the forebrain periventricular layer, the extent of cell damage was declining towards the olfactory bulbs (Fig. 3).

Discussion

BSH is a hydrophilic molecule which does not cross easily the hematoencephalic barrier (HEB) (Abe *et al.* 1988, Clendenon *et al.* 1990, Stragliotto and Fankhauser 1995). Incomplete functioning of HEB in brain tumor leads to preferential entrance of the parenterally administered BSH into the tumor cells. As shown in tissue cultures, the brain tumor cells *per se* also accumulate more BSH than normal glial cells (Mareš *et al.* 1992). In 1-week-old rats used in this study, the HEB has not yet been fully developed. Nevertheless, to achieve a sufficient and controlled levels of BSH, as well as to prevent the neutron-capture reaction in the remaining parts of the body, we preferred intracranial administration of the drug shown to provide massive penetration of the HEB-impermeable large molecules into the parenchyma from the drug-filled ventricular system (Mareš *et al.* 1984).

The data showed that neutron beam *per se* (i.e. irradiation without BSH) caused only a small damage limited to regions composed of highly radiosensitive dividing cells (Mareš and Brückner 1976). The regions with more differentiated and postmitotic cells were left morphologically unaffected. In comparison to this, irradiation in the presence of boron-10 induced a significantly more extensive damage already after 41 cGy-Eq (5 min irradiation time). Higher doses (10 and 20 min irradiation, 82 and 164 cGy-Eq) have destroyed also some cells in the more differentiated brain regions, specifically the internal granular layer and the medulla of the cerebellum and superficial layers of the cerebral cortex. The cell damage displayed features of programmed cell death (apoptosis) similar to those described, for instance, in the cerebellum of immature rats treated with Cisplatin, a DNA binding cytostatic (Mareš *et al.* 1986, Scherini *et al.* 1987, Biggiogera *et al.* 1990).

It is to be pointed out that dividing cells are generally very sensitive to ionizing radiation. As a consequence, a small number of cells in the external granular layer of the cerebellum and the subependymal layer of the forebrain were damaged in animals without BSH. This effect may be attributed to the contamination of epithermal neutron beam by fast neutrons and the effect of gamma rays formed during the process of thermalization of neutrons in the irradiated tissue. Contribution of the neutron-capture reaction occurring on the traces of naturally occurring boron-10, and occasionally also atoms of nitrogen and hydrogen is to be considered as well (Kliegel 1980, Barth *et al.* 1990). As demonstrated, these unwanted side effects were minimal and limited to a small number of the most sensitive dividing cells. A more extensive damage of dividing cells, as well of a certain number of cells in the more differentiated parenchyma, appeared only after amplification of the radiation effects by a neutron capture reaction on BSH.

It is noteworthy that dividing cells in the brain of adults, including man, are extremely rare (Mareš and Lodin 1974). As a consequence, adult brain is several times more resistant to radiation than that of immature individuals. Therefore, we can reasonably assume that normal brain parenchyma of adult patients treated by the epithermal beam of LVR-15 reactor would be affected much less than it was observed in control animals in our study. In the dividing tumor cell population we can, however, expect the degree of destruction similar to that observed in the germinative regions of the cerebellum and the forebrain of the immature rats. Dividing cells represent, however, only a small part the whole population of tumors and effective killing of the non-cycling cells will require higher neutron fluxes.

Finally, it is to be pointed out that partial destruction of the germinative compartments of the fetal rat brain by X-rays requires the doses of 100–200 cGy (Bruckner *et al.* 1980). In the present study, comparable lesions in the cerebellum appeared due to neutron-capture reaction already after 40 cGy. This is in agreement with 2 to 3 times higher biological effectiveness of alpha particles formed during interaction of boron-10 with thermal neutrons (Gabel *et al.* 1984, Fukuda *et al.* 1987). In the case of clinical applications of BNCT, this enhancement would be limited to BSH-enriched tumor cells while normal parenchyma would be exposed only to low background radiation represented by slow neutrons.

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