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DELAYED EFFECTS OF NEUTRON IRRADIATION ON CENTRAL NERVOUS
SYSTEM MICROVASCULATURE IN THE RAT *

J.H. Goodman, J.M. McGregor, N.R. Clendenon, W.A. Gordon, and
A.J. Yates, R.A. Gahbauer, R.F. Barth, R.G. Fairchild*

Ohio State University College of Medicine
Columbus, Ohio

*Brookhaven National Laboratories
Upton, New York

INTRODUCTION

Pathologic examination of a series of 14 patients with malignant gliomas treated with BNCT showed well demarcated zones of radiation damage characterized by coagulation necrosis. Beam attenuation was correlated with edema, loss of parenchymal elements, demyelination, leukocytosis, and peripheral gliosis. Vascular disturbances consisted of endothelial swelling, medial and adventitial proliferation, fibrin impregnation, frequent thrombosis, and perivascular inflammation. Radiation changes appeared to be acute and delayed. The outcome of the patients in this series was not significantly different from the natural course of the disease, even though two of the patients had no residual tumor detected at the time of autopsy. The intensity of the vascular changes raised a suspicion that boron may have sequestered in vessel walls, resulting in selectively high doses of radiation to these structures (Asbury et al., 1972), or that there may have been high blood concentrations of boron at the time of treatment. The potential limiting effects of a vascular ischemic reaction in Boron Neutron Capture Therapy (BNCT) prompted the following study to investigate the delayed response of microvascular structures in a rat model currently being used for pre-clinical investigations.

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DISCLAIMER

Table 1. BNCT Dosimetry of Animals Examined

Specimen	Neutron Fluence	Boron
1.	8X10(12) N/cm ²	none
2.	2X10(13) N/cm ²	none
3.	6X10(12) N/cm ²	50 mg/kg
4.	8X10(12) N/cm ²	50 mg/kg

MATERIAL AND METHODS

Thirty CD Fischer 334 adult male rats were studied following exposure to thermal neutron beam irradiation provided by the Brookhaven National Laboratory Medical Reactor. B10 enriched sodium mercaptoundecahydrododecaborate (NA₂H₁₂B₁₁SH), 50 mg/kg IV (26.9mg B10/kg) was given to one half of the rats 17-18 hours before treatment. Rats were immobilized in a fixed head-holder, and right cerebral hemispheric irradiation was focused through a 1-cm diameter port centered 3mm to the right of midline, and 7mm anterior to the external meatal line. The rats were divided into groups based on the thermal neutron fluence received. Irradiation was performed in single fractions of various durations up to 8 minutes to obtain the desired fluences. Selected specimens were obtained for study of the late effects of BNCT (Table 1).

Nineteen months after treatment those rats selected for electron microscopy were anesthetized with ketamine (100mg/ml) in a ketamine/xylazine mixture of .12 ml/.1 mg given in the dose 0.1 ml/100 gm body weight by i.p. injection. They were killed by whole body perfusion fixation, using half-strength Karnovsky's solution, consisting of 2% Paraformaldehyde and 2.5% Glutaraldehyde in 0.12 molar Sorensen's phosphate buffer, pH 7.4, containing 60 mM sucrose and 0.5 mM calcium chloride. Fixation was performed via intra-aortic cannulation at a perfusion pressure of 140 cm. of water. This procedure provided adequate fixation of the CNS. The brains were removed and serial coronal sections 1mm thick were cut. Tissue blocks 1mm³ were obtained from the right caudate nucleus, corpus callosum and cerebral cortex.

Tissue blocks for electron microscopy were fixed for one hour at room temperature in the half-strength Karnovsky's solution. Samples were rinsed several times in a 0.1 molar phosphate buffer with 0.1 molar sucrose at 4 degrees centigrade and placed in 1% osmium tetroxide in buffer for 2 hours at 4 degrees centigrade. The tissues were rinsed in cold buffer and stored 24 hours at 4 degrees centigrade, then subsequently dehydrated in graded ethanol and two changes of propylene oxide. The tissues then were infiltrated with

spurr resin (1:1 spurr/propylene oxide) for 1 hour, rinsing twice in spurr resin, 1 hour each, and embedding. The tissue was post-stained with lead citrate and uranyl acetate before analysis. Electron micrographs were obtained on Phillips EM 300 and EM 301 electron microscopes.

Whole mount coronal sections were processed for histological study and stained with hematoxylin and eosin, phosphotungstic acid-hematoxylin, Bodian and luxol fast blue.

Adult control rats, not matched for age, received neither irradiation nor boron compound and were analyzed in the same manner.

Fig. 1. Rat caudate nucleus. Photomicrographs taken of right caudate nucleus beneath the neutron radiation port. (A) normal control. (B) rat treated with BNCT (specimen 4). Similar vascular patterns are noted. Reactive changes including gliosis and perivascular infiltration are absent in irradiated tissue. Hematoxylin and eosin stain. Scale represents 0.1mm.

RESULTS

The observations reported focus solely on the microvascular architecture and perivascular structures in gray matter.

Light Microscopy

Coronal whole brain sections were used to correlate histopathology with samples taken for electron microscopy. Cortical gray matter and caudate nucleus beneath the beam port were examined following histochemical staining. Perfusion fixation resulted in distended vessels uniformly distributed throughout the tissues examined. Those animals undergoing neutron irradiation alone and neutron irradiation with boron showed similar vascular patterns as seen in the controls. Uniform thickness of capillary endothelium, lack of gliosis, and no evidence of tissue necrosis characterized all samples (Fig. 1). PTAH-stained sections did not show evidence

Fig. 2. Electron micrographs of capillaries from right caudate nucleus. (A) normal control. (B) rat treated with BNCT (specimen 4). Endothelial cytoplasm and basal laminae display uniform thickness and regular margins. Junctional complexes are noted. Intracytoplasmic organelles have a uniform appearance in both specimens. Pericyte processes maintain their perivascular relationships. Scale represents 1μ .

of gliosis that might have been related to direct tissue effects of radiation or secondary vascular ischemic changes.

Ultrastructural observations

The capillary endothelium appeared to have a uniform thickness with the usual number of intracytoplasmic organelles. Occasional pinocytotic pits and vesicles were encountered. Endothelial junctional complexes demonstrated sites of increased cytoplasmic density. The endothelial nuclei appeared morphologically normal (Fig. 2). The basal lamina was of uniform density and thickness, and completely encircled all vessels. Pericytes and pericyte processes were completely enclosed within the basal lamina. In larger vessels, collagen filaments were noted. Those larger vessels considered to be precapillary arterioles and postcapillary venules contained both collagen and smooth muscle cells. Pericytes

Fig. 3. Right caudate nucleus from a rat treated with BNCT (specimen 4). Two pericyte nuclei (n) are seen between the capillaries. The basal lamina completely encloses the cytoplasm of the pericyte (arrows). Note the numerous pleomorphic dense bodies within the cytoplasm, consistent with the phagocytic activity of these cells. Scale represents 1 μ .

contained within the basal lamina of irradiated animals showed occasional accumulations of numerous dense bodies within the cytoplasm consisting of either lipid or condensed exogenous protein (Fig. 3). A glial investment consisting of clear and fibrous astrocytes surrounded the capillaries, arterioles, and venules.

There were no obvious differences between the control samples and radiated animals in the endothelium, basal lamina, or perivascular glia. Evidence of pathologic changes seen in pericytes consisted of vacuolization within membrane bound organelles in the radiated animals.

DISCUSSION

Radiation damage to the nervous system can be related to dose and latency, with damage appearing as late as 2 years after radiation to the spinal cord of the rat (Hubbard and Hopewell, 1978). Radiation of normal rat brain with 2000 cGy causes progressive vascular alterations over a period of two years, consisting of increased vascular density and telangiectasias (Reinhold and Hopewell, 1980). CNS vascular responses to varying doses and latencies are shown by antipyrine extraction studies to be progressive over several months to one year (Moustafa and Hopewell, 1979). Late focal vascular occlusions in the irradiated rat brain have been reported (Hopewell, 1974). Ultrastructural evidence of capillary tolerance to BNCT in the cerebral cortex of the dog was reported by Al-Samarrai et al. (1975).

Our current studies involve a group of animals treated with neutron fluences from 6×10^{12} to 2×10^{13} N/cm². Animals receiving a higher radiation dose of 4×10^{13} N/cm² do not survive and typically die within two weeks. The dosages tested are being used to evaluate BNCT in a nitrosourea-induced glioma model, and this report indicates that late radiation vasculopathy at such doses is minimal. Pericyte phagocytic activity is a nonspecific reaction that occurs following pathologic conditions and has been observed 28 days after 1000 rad neon radiation in the mouse (Polak et al., 1982), and following 6000 rad alpha-particle irradiation to rat cerebral cortex (Maxwell and Kruger, 1965).

Vascular complications associated with BNCT clinically, and the known delayed CNS radiation effects justify further studies to establish normal tissue tolerances. The potentially devastating late vascular complications must be avoided. Cross-species testing to confirm tolerance limits in the dog and in primates will aid in planning clinical protocols. Further investigations will provide insights into the vascular mechanisms by which BNCT provides therapeutic effects, to determine the optimal parameters for future clinical trials.

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