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The Postradiation Demyelination of the Optic Nerve

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UDC 616.833.115-02:615.849.1]-07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 118, № 11, pp. 551-554, November, 1994
Original article submitted February 28, 1994

Neutron and x-radiations induce focal dose-dependent demyelination of the optic nerve. Myelin sheaths are more radiosensitive than axis cylinders. It is shown that phagocytic activity of fibrillary astroglia and endocytosis of myelin by altered axons play a key role in postradiation demyelination.

Key words: *neutrons; x-rays; optic nerve*

Damage to myelin sheaths under the impact of ionizing radiation has been reported in various regions of the brain and spinal cord [5,7]. However, the mechanisms of this phenomenon are not completely understood and, as a rule, are attributed to radiosensitivity of myelin-producing oligodendrocytes [1,6].

In view of the high probability of radiation injury of the optic nerve occurring in oncoradiology [4], the aim of the present investigation was to identify the role of glial and neuronal elements in demyelination of the optic nerve after exposure to sparse and dense ionizing radiation.

MATERIALS AND METHODS

The study was performed on 45 guinea pigs of both sexes with an initial weight of 400-450 g, 25 of which were exposed to a single whole-body x-raying with a dose of 4.5 Gy ($LD_{50/30}$) using an RUM-17 apparatus (40 cm focal distance, 0.5 mm Cu filter, dose rate: 0.64 Gy/min) and 20 animals were the control. The right eye of 45 male chinchilla rabbits (2-2.5 kg weight) was exposed to fractionated neutron radiation with a dose of 1.5 Gy per fraction twice a week (Monday and Friday) according to the treatment protocol for cancer patients used at the Oncological Institute of the Tomsk Science Center, Russian Academy of Medical Sciences. Fast neutrons (6.2 MeV) were produced by bombardment of a beryllium target in a U-120 cyclotron (proportion of gamma-quanta 8-10%, dose rate 0.15 Gy/min, 4×6 cm field). Forty-four control rabbits were exposed to sham irradiation and kept under the same conditions as the treated animals with the usual 24-h light regimen in the vivarium. Decapitation of the animals and collection of the material (the optic nerves) were performed 1, 5, 10, 25, and 60 days after whole-body x-raying and after local neutron exposure 1 day and 6 months later if the total doses attained 3 and 15 Gy, 1, 10, and 30 days and 6 months later at 7.5 Gy, and after 24 h at 40.5 Gy. Bioptates from 16 cancer patients with paraorbital tumors that had not spread to the eyeball and optic nerve were studied, 12 of these patients having received a course of radiotherapy with a total focal dose of 9-10.5 Gy prior to surgery. With

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0007-4888/94/0011-1249$12.50

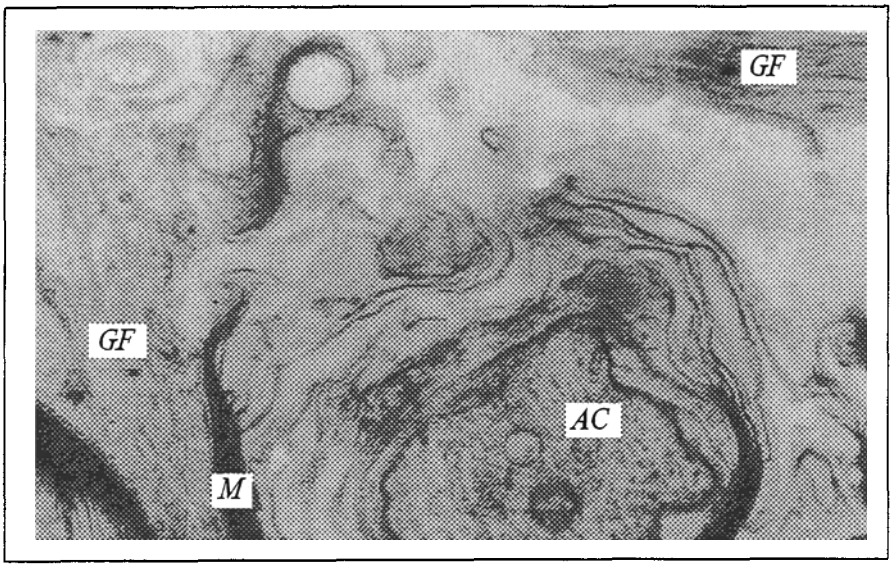

Fig. 1. Focal demyelination of a rabbit optic nerve fiber on the 30th day after fractionated neutron irradiation of the eye with a total dose of 7.5 Gy. *AC*: axis cylinder; *M*: myelin; *GF*: gliofilaments. ×19,000.

shielding taken into account, the total dose ranged from 1.5 to 3 Gy per.

For electron microscopy fixation was performed by immersion and perfusion with 2.5% glutaraldehyde in cacodylate or phosphate buffer (pH 7.4), with postfixation in 1% osmium tetroxide followed by embedding in araldite. Ultrathin sections were obtained with an LKB-III ultratome, stained with uranyl acetate and lead citrate, and examined under a JEM-100CX electron microscope. The percentage of altered myelin sheaths and axis cylinders was calculated per 200 fibers of every optic nerve as well as the mean number of microtubules and neurofilaments in axis cylinders of equal diameter (1±0.05 μ). Statistical processing was performed using the Student *t* test.

RESULTS

The pathomorphological changes used as the ultrastructural criteria for quantitative assessment of the damage to the fiber components in the optic nerve were as follows:

a) focal demyelination manifested in local stratification and destruction of the myelin sheath; with dissolving and phagocytosis of myelin layers;

b) dark-type degeneration, "mummification" and lumpy disintegration of the axis cylinder;

c) combination of demyelination and degeneration of the axis cylinder.

Alternative changes of the optic nerve appeared after neutron irradiation have a dose and time dependence, the glial component (myelin sheath) suffering to a greater extent than the axis cylinder (Table 1). For example, focal demyelination on the first day after irradiation of rabbit eyes at doses of 3 and 7.5 Gy is not substantial or statistically significant in total doses of 15 and 40.5 Gy. Development of demyelination 30 days after irradiation in a dose of 7.5 Gy resulted in marked changes as compared to the control. In the long term (6 months) demyelination becomes pronounced even at 3 Gy.

The study of the optic nerves from patients with paraorbital tumors at a late term (18-20 months after neutron therapy) attests to an increase of the number of nerve fibers with demyelination signs to 7.3±1.7% versus 3.2± 1.3% in the control ($p<0.05$).

After whole-body x-raying at 4.5 Gy the number of focal demyelinated nerve fibers increases to 11-15% (5-6% in the control, $p<0.05$) at 10-25 days in the guinea pig optic nerve.

Cell-to-cell interactions and the role of the glial and neuronal elements are of interest for the elucidation of the demyelination mechanisms long discussed in the neuromorphological literature [1,2,8]. The model exposed to neutron impact with doses of 3 and 7.5 Gy shows the reactive changes in oligodendrocytes which were manifested in the dilation and fragmentation of cisternae of the endoplasmic reticulum and in changes in the number of polysomes and in the degree of chromatin condensation. The number of lysosomes, phagosomes, and myelinlike bodies increases in some oligodendrocytes in the late periods, indirectly attesting to the phagocytic activity of these cells. After treatment in doses of 15 and 40.5 Gy many oligodendrocytes become pyknomorphous.

However, the specific variety of fibrillary astroglia identified under the electron microscope due to the gliofibrillary apparatus plays the most important role in demyelination of the optic nerve. The processes of astrocytes situated between nerve

fibers and forming a kind of endoneurium are characterized by a low electron density and contain fragments of captured membrane plates of myelin close to the demyelination focus (Fig. 1).

Endocytosis of myelin sheath fragments by the axis cylinder (Fig. 2) noted after the ionizing impact becomes more and more significant in the mechanisms of demyelination as the dose of radiation and amount of time elapsing increase. It should be noted that there is a virtual absence of microtubules in such axis cylinders, which points to a similarity of endocytosis these axons to the analogous process reported previously in dendrites of the central nervous system [3]. Neuronal processes may actuate the reliable ancient (phylogenetic) mode of trophics through endocytosis under conditions of the destruction of the neurotubular apparatus and, correspondingly, disturbance of the axonal transport leading to nutrient deficiency. However, the compensatory capacity of endocytosis is limited and the overload of the neuroplasm with myelin fragments leads to axon degeneration and death.

The degenerative changes of axis cylinders are less pronounced than the damage to myelin sheaths, and the rising percentage of damaged axons becomes significant only at doses of 15 and 40.5 Gy (Table 1). In addition to the dramatic destruction of axis cylinders, reactive shifts in the ultrastructure of mitochondria and in the number of microtubules were found as early as the first day after neutron irradiation in doses of 3 and 7 Gy. When the dose is increased to 40.5 Gy, the mean number of microtubules drops to 21.7±2.4 (30.6±2.9 in the control, $p<0.05$) in axis cylinders of 1±0.5 μ diameter in the retrobulbar part of the optic nerve and to a greater extent at the level of the chiasma to 8.4±3.9 (28.1±3.4, $p<0.01$ in the control). It is noteworthy that at the same time the number of neurofilaments

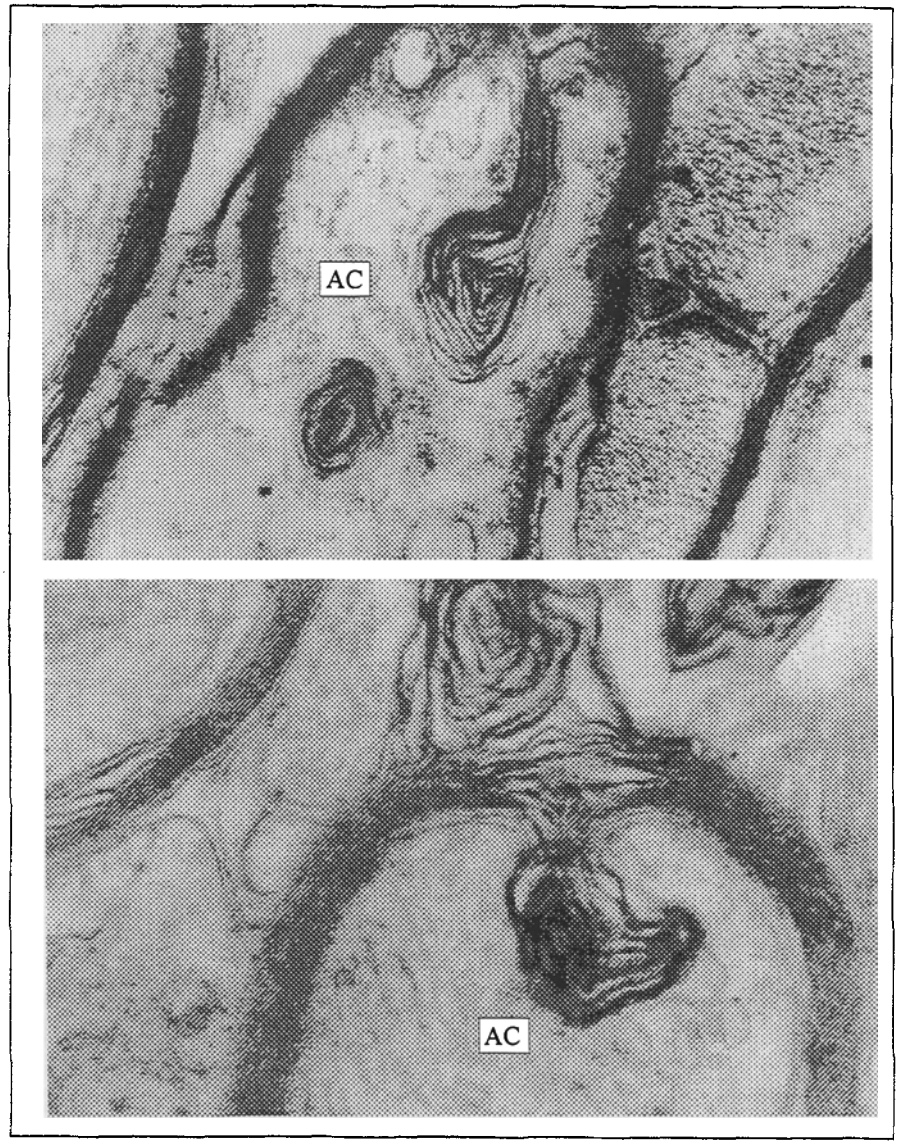

Fig. 2. Endocytosis of myelin by axis cylinders of the optic nerve: *a*) capture of myelin by axis cylinder on the 30th day after local neutron irradiation with a total dose of 7.5 Gy; *b*) concurrent captures of myelin by axis cylinder and glia on the 10th day after whole body x-raying in a dose of 4.5 Gy. *AC*: axis cylinder. ×29,000

TABLE 1. Quantitative Ultrastructural Characteristics of Pathomorphological Changes of the Optic Nerve at Different Times after Neutron Irradiation

Dose and times after treatment	Percentage of altered nerve fibbers		
	with focal demyelination	with degeneration of axis cylinder	with combined demyelination and degeneration of axis cylinder
Control I	6.5±0.88	1.0±0.29	0.1±0.09
3 Gy, 1 day	7.5±1.85	1.6±0.90	0.1±0.10
3 Gy, 6 months	15.1±1.96***	1.9±0.66	0.3±0.30
Control II	0.0±0.94	1.1±0.30	0.1±0.07
7.5 Gy, 1 day	9.2±2.18	2.2±1.02	0.3±0.20
7.5 Gy, 10 days	10.3±2.06	2.0±0.82	0.1±0.10
7.5 Gy, 30 days	16.9±4.17*	1.4±0.38	0.3±0.14
7.5 Gy, 6 months	28.5±4.00*	1.8±0.66	0.2±0.20
Control III	6.2±1.02	0.9±0.09	0.2±0.11
15 Gy, 1 day	10.8±1.77	3.2±0.80**	0.4±0.25
40.5 Gy, 1 day	12.8±2.15**	2.8±0.46*	2.2±0.46***
40.5 Gy, 1 day (chiasma)	12.3±1.64**	3.4±0.69**	9.0±1.17***

Note. One, two, and three asterisks show $p<0.05$, $p<0.01$, and $p<0.001$, respectively, as compared to the corresponding control.

also increases to 83.2±6.8 (61.5±5.3 in the control, $p<0.05$) and at the chiasma level to 155.4±17.5 (65.3±8.7 in the control, $p<0.01$).

The combination of demyelination and degeneration of the axis cylinder is less common than the separate destruction of each fiber component, and a reliable dynamics of this index was noted only at a dose of 40.5 Gy. The combined injury of myelin sheath and axon is more pronounced at the chiasma level than in the retrobulbar segment of the optic nerve (Table 1).

Vascular disorders and perivascular sclerosis in the optic nerve manifest themselves at summated neutron doses of 7.5 Gy and aggravate nerve fiber damage.

Thus, neutron and x-radiations induce focal dose-dependent demyelination of the optic nerve. Myelin sheaths are more radiosensitive than axis cylinders. Phagocytic activity of fibrillary astroglia and endocytosis of myelin by altered axons play a key role in the mechanisms of postradiation demyelination.

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