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Effect of Radiation with Low Doses Fast Neutrons and High Energy Oxygen Ions on the Rat Choroid Plexus Blood Vessels

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In the present study the morphometric analysis has been used to investigate the changes of the rat choroid plexus blood vessels. The applied irradiation provoked statistically significant changes in the rat choroid plexus blood vessels. These data provided evidence that the effect on the blood vessels in the delay period after irradiation can be induced in the rat choroid plexus by single doses of 1.0 Gy fast neutrons and oxygen ions. We suggest that these changes may be related with alteration of cerebrospinal fluid secretion and transcellular and absorbtion-transport functions of the rat choroid plexus.

Key words: rat choroid plexus blood vessels, morphometry, low doses of fast neutrons and oxygen ions irradiation.

Introduction

The choroid plexuses are specialized highly vascular anatomycal structures which protrude into the lateral ventricle, as well as in the third ventricle and fourth ventricle. The surface of the choroid plexus consist of numerous villi each covered with single layer of epithelial cells surrounded by vascular connective tissue cells [7]. As a secretory source of vitamins, peptides and hormones for neurons, the choroid plexus provides substances for brain homeostasis [4]. Most blood vessels in the plexus choroideus are wide-calibre (approximately 15 μm) fenestrated capillaries [6]. Irradiation with therapeutic doses may result in the development of early and late effects in normal tissues. Extensive experimental studies have shown a clearly defined association between vascular damage and the development of late radiation effects, however, the exact role played by vascular lesions remains uncertain [3]. The aim of the present study is to investigate the morphometrical changes of the blood vessels of the rat choroid plexus after exposure to low doses of fast neutrons and high-energy oxygen ions irradiation.

Materials and Methods

Three months aged female Wistar rats were exposed to fast neutrons (n=3) to 1.5 MeV at the dose of 1.0 Gy and single dose of 10^4 particles/cm^2 of oxygen ions (n=3). Experiments were performed in the impulsive reactor and the synchrophasotron of the Joint Institute of Nuclear Research in Dubna, Russia. Three months after whole-body irradiation experimental animals and 6 months aged control rats (n=4) were perfused intracardially with 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodilate buffer [5]. Extracted choroid plexuses were postfixed in 1% OsO4 and embedded in Durcupan. The semithin sections (1μm) were stained with 1% toluidine blue for morphometric measurment and examined under Light microscope Carl Zeiss Jena.

Morphometric analysis

We obtained morphometric data from the light microscope at 1000 × magnification using a square grid system [9] calibrated for linear measurment in μm and area measurment in μm^2 (625 grid points). We measured the relative number of blood vessels and luminal diameter and area of the blood vessels divided in four subgroups. The luminal diameter was measured as perpendicular distance across the maximum chord axis of each vessels.

Statistical analysis

Results are reported as mean values ± SEM and as relative part in percentage. We evaluated data from control rats and irradiated rats by using the Student's t-test for independent comparisons.

Results

Changes in the relative number of all blood vessels and vessels divided in four subgroups were determined after irradiation with a single dose of 1.0 Gy fast neutrons and oxygen ions in comparison to control rats. These findings are shown in Figures 1 and 2 and Tables 1 and 2. The significant changes three months after fast neutrons irradiation were approximately 13% reduction (p<0.001) in the number of vessels of 5-7 μm in diameter and 33% reduction (p<0.001) in vessels of 7-16 μm in diameter. A significant increase of 32% was seen in the number of large vessels of 16-30 μm

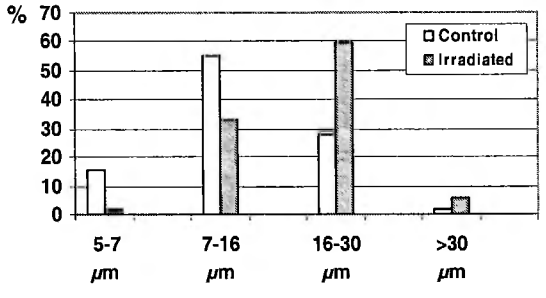

Fig. 1. Comparison of morphometric data of choroid plexus blood vessels of control rats and fast neutrous irradiated rats (% — relative parts)

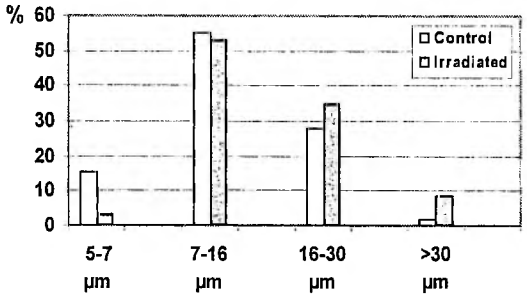

Fig. 2. Comparison of morphometric data of choroid plexus blood vesse of control rats and high-energy oxygen ions irradiated rats (% — relative parts)

($p<0.001$) and 3% in vessels >30 μm in diameter. Similar changes were determinated after irradiation with a single dose of high energy oxygen ions in comparison to control rats: approximately 12% reduction ($p<0.001$) in the number of vessels of 5-7 μm in diameter and 2% reduction in vessels of 7-16 μm in diameter. A significant increase of 7% was seen in the number of large vessels of 16-30 μm ($p<0.01$) and >30 μm (<0.01) in diameter after irradiation with oxygen ions.

Significant changes were not seen in luminal diameter (mean luminal diameter of neutrons irradiated rats — 18.80 ± 0.93 μm and oxygen irradiated rats — 18.87 ± 0.93 μm and mean luminal diameter of control rats — 18.50 ± 0.94 μm) and luminal

T a b l e 1. Morphometric data of choroid plexus blood vessels of control rats and fast neutrons irradiated rats (luminal diameter in μm, luminal area in μm²)

Blood vessels	Control rats		Irradiated rats	
	Luminal diameter ± SEM	Luminal area ± SEM	Luminal diameter ± SEM	Luminal area ± SEM
5–7 μm	7.37±0.24	100.92±2.84	7.50±0.20	87.50±12.50
7–16 μm	11.16±0.41	174.13±10.52	12.64±0.74	168.75±17.10
16–30 μm	21.56±1.26	401.28±30.59	20.56±1.04	369.53±36.45
>30 μm	33.87±1.87	731.25±40.49	34.37±1.76	791.66±40.82
Number of measurments	242	242	159	159

T a b l e 2. Morphometric data of choroid plexus blood vessels of control rats and high-energy oxygen ions irradiated rats (luminal diameter in μm, luminal area in μm²)

Blood vessels	Control rats	Irradiated rats
	Luminal diameter ± SEM	
5–7 μm	7.37±0.24	7.05±0.23
7–16 μm	11.16±0.41	11.55±0.59
16–30 μm	21.56±1.26	21.73±1.62
>30 μm	33.87±1.87	34.73±1.59
	Luminal area ± SEM	
5–30 μm	199.08±17.51	212.22±18.13
Number of measurements	242	148

area (mean luminal area of neutrons irradiated rats — 354.36 ± 26.71 μm² and mean luminal area of control rats — 351.89 ± 20.52 μm²) of all blood vessels.

Discussion and Conclusion

In the present study it was estimated that the relative part of the capillaries, i.e. vessels <16 μm in diameter were 70.66% in control rats, and 34.86% in fast neutrons and 56.34% in oxygen ions irradiated rats. The initial loss of capillaries and the increase in number of larger vessels in plexus choroideus three months after irradiation were consistent with the effects attributed to regeneration in the choroid plexus. Ultrastructural and morphometrical changes were reported previously in the cytoplasm of endothelial cells of fenestrated capillaries and epithelial cells of this experimental model [1, 8]. Most of the choroid plexus epithelial cells exhibited ultrastructural signs of increased absorbing and transport activity. The augmented transport activity was evident from a large number of micropinocytotic vesicles in cytoplasm of the endothelial cells of fenestrated capillaries. This increased intensity of the cellular transport relates, probably, with early post-exposure edema or subserves elimination of toxic substances consequent to radiation exposure. From the morphometric investigation in the previously our study it was established that the nuclear, cytoplasmic and cell area of the light and dark epithelial cells diminished after exposure to fast neutrons. These changes may be indicative of compensatory reactions in the organism following radiation exposure. Experimental study have shown a significant reduction in the number of blood vessels > 16 μm in diameter and atrophy of the choroid plexus epithelial cells only after 25 Gy of X-rays [2, 3].

In conclusion, it can be pointed out that the applied irradiations provoked significant changes in rat choroid plexus blood vessels. These results clearly demonstrate that the effect on blood vessels after irradiation can be induced in the choroid plexus by single dose of 1.0 Gy fast neutrons and oxygen ions. We suggest a hypothesis that the vascular damage is predominant factor leading to development of late effects in irradiated normal tissues.

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