*Original Article*

# ULTRASTRUCTURAL AND MORPHOMETRIC CHARACTERISTICS OF RAT CHOROID PLEXUS EPITHELIAL CELLS AND BLOOD VESSELS AFTER LOW DOSES IONIZING IRRADIATION

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## Summary

In the present study were carried out ultrastructural and morphometrical investigations of the light and dark rat choroid plexus epithelial and endothelia cells after low doses of ionizing irradiation of high energy oxygen ions, neutrons and gamma rays. The total-body exposures to low doses fast neutrons and gamma rays provoked similar ultrastructural alterations in the rat choroid plexus. Most of the epithelial cells exhibited ultrastructural signs of increased transcellular transport of substances. Ultrastructural changes of the choroid plexus epithelial cells after exposure to high energy oxygen ions included increased absorption-secretion activities and stimulation of the cell metabolism that could be a compensatory reaction to the changes shortly after the experimental exposure. The applied irradiation provoked statistically significant changes in the rat choroid plexus epithelial cells. The obtained ultrastructural and morphometrical data after total-body irradiation of rat with low doses have shown that changes of the epithelial and endothelial cells of the plexus choroideus are more marked after irradiation with oxygen ions and neutrons in comparison with gamma rays. 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, gamma rays and oxygen ions. We suggest a hypothesis that the vascular damage is predominant factor leading to development of late effects in irradiated normal tissues.

**Key words:** rat choroid plexus epithelial cells and blood vessels, ultrastructure and morphometry, low doses high energy oxygen ions, neutrons and gamma rays

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## Acknowledgment

This work was supported by the National Science Fund Ministry of Education and Science (Contract № H11B-66), Sofia, Bulgaria

## Introduction

Humans, animals and plants have been exposed to natural radiation since the creation of life. The choroid plexuses are specialized highly vascular anatomycal structure which protrude into the lateral ventricle, as well as in the third ventricle and fourth ventricle. The surface of the choroid plexus consists of numerous villi each covered with single layer of epithelial cells surrounded by vascular connective tissue cells [1]. As a secretory source of vitamins, peptides and hormones for neurons, the choroid plexus provides substances for brain homeostasis [2]. Most blood vessels in the plexus choroideus are wide-calibre (approximately 15μm) fenestrated capillaries [3].

The ***aim*** of the present study is to investigate the ultrastructural and morphometrical changes of the rat choroid plexus epithelial cells and blood vessels after low doses ionizing irradiations.

## Materials and Methods

Three-months aged female Wistar rats were divided into four group: I group - irradiated with single dose of $10^4$ particles/cm² of oxygen ions (n=3), II - group irradiated with fast neutrons (n=3) to 1.5 MeV at the dose of 1.0 Gy, III group - irradiated with gamma rays $Co^{60}$ (n=3) at the dose of 1.0 Gy and IV group - control six months rats (n=4). Three months after irradiation the animals were intracardially perfused. Extracted choroid plexuses were postfixes in 1% $Os_4$ in 0.2 M cacodilate buffer, dehydrated through graded ethanol and embedded in Durcupan and examined with JEOL JEM 1200EX transmission electron microscope.

Morphometric data were obtained from semithin sections using a square grid system [4, 5] calibrated for linear measurement in μm and area measurement in ìm² (625 grid points). All values were expressed as mean ± SEM, and statistically analyzed by Student t-test using statistical package (STATISTICA, ver.6, Stat-Soft Inc., 2001).

## Results

Choroid plexus of the brain is an ideal model for studying the development of radiation damage due to a close contact between vascular and epithelial cells, which normally have very slow turnover. In contrast to control rats on the apical surface of the epithelial cells of the rat choroid plexus 3 months after irradiation with high energy oxygen ions were seen cytoplasmic protrusions. The microvilli were elongated and dilated. On the apical epithelial surface were seen intraventricular macrophages, many vacuoles in the basal epithelial part, and many complex infoldings of the intercellular basal lamina (Fig. 1). The epithelial nuclei were oval with small invaginations. In the epithelial cytoplasm there are seen well defined Golgi apparatus, dense bodies, vesicles and multivesicular bodies. Other characteristic ultrastructural change of the choroid plexus epithelial cells was the presence of vacuoles, containing glycogen granules at the apical epithelial cytoplasm. In the epithelial cytoplasm of the rat choroid plexus 3 months after irradiation with fast neutrons were seen well defined Golgi apparatus, coated vesicles, pinocytotic vesicles and multivesicular bodies (Fig. 2). Most characteristic ultrastructural changes of the rat choroid plexus 3 months after irradiation with gamma rays were elongated mitochondria, localized at the apical part of the epithelial cells and many pinocytotic vesicles on the basolateral intercellular junctions as well as in the endothelial cells of the capillaries, and many epithelial cells possessed two nuclei (Fig. 3, 4).

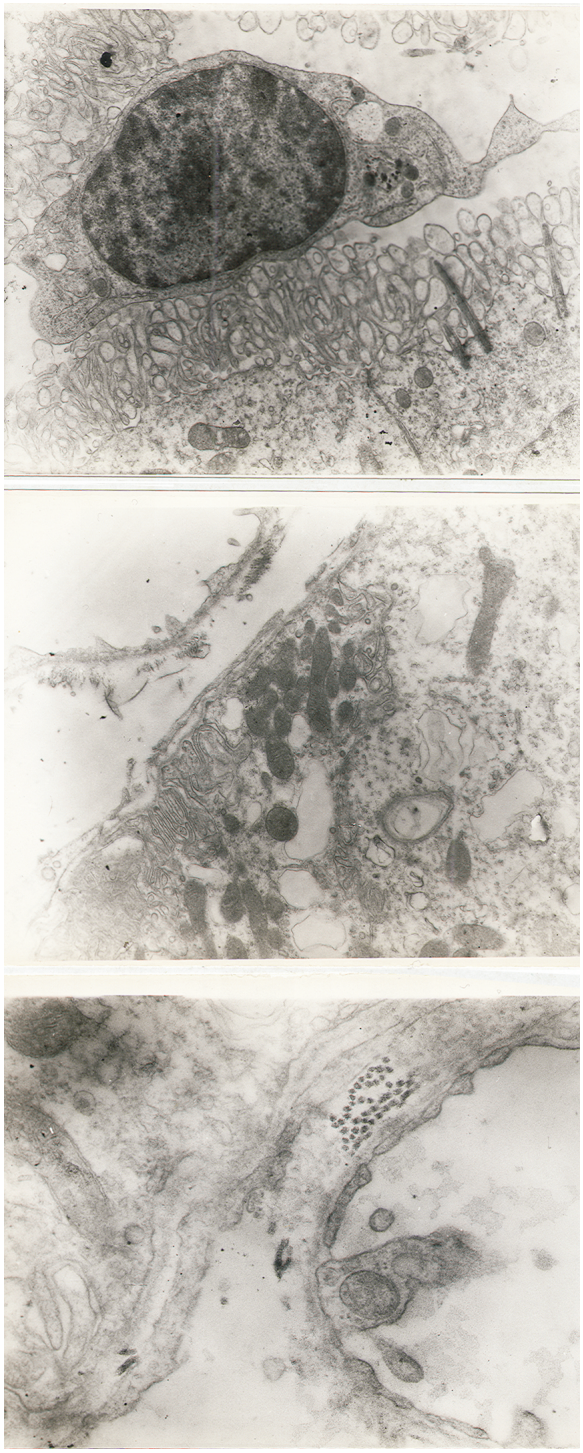

**Fig. 1**. Epithelial cell of the rat choroid plexus 3 months after irradiation with high energy oxygen ions.
A/ Intraventricular macrophage, X 6 000;
B/ Basal part - there are many vacuoles; the intercellular basal lamina with many complex infoldings, X 7 500;
C/ Fenestrated capillary, X 30 000

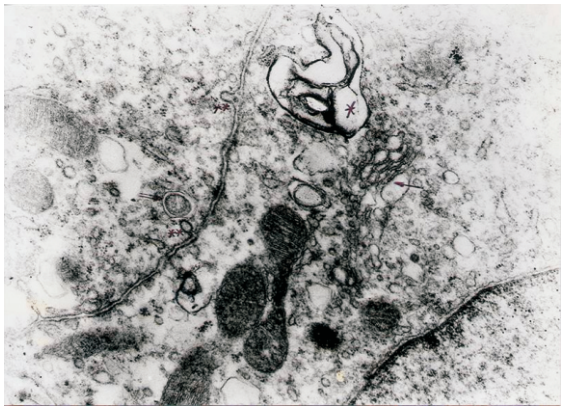

**Fig. 2.** Epithelial cells of the rat choroid plexus 3 months after irradiation with fast neutrons. In the epithelial cytoplasm were seen well defined Golgi apparatus (->), coated vesicles (=>), multivesicular bodies (*), pinocytotic vesicles (**) X 15 000 B/ There are elongated mitochondria in the cytoplasm (->) X 15 000

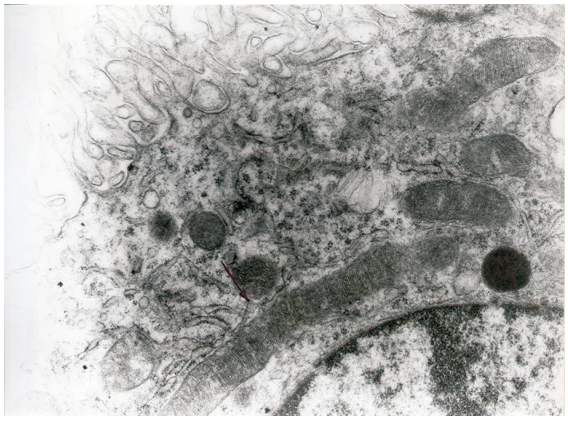

**Fig. 3.** Apical part of the epithelial cells of the rat choroid plexus 3 months after irradiation with gamma rays. There are elongated mitochondria in the cytoplasm. X 15 000

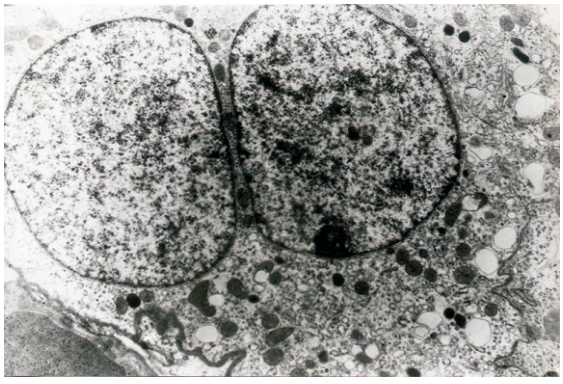

**Fig. 4**. Binuclear epithelial cell of the rat choroid plexus 3 months after irradiation with gamma rays. X 5 000

The applied irradiation with oxygen ions, fast neutrons and gamma rays provoked statistically significant changes of the morphometrical parameters of the light and dark epithelial cells in comparison with control ones. The nuclear area of the light epithelial cells diminished with 4.65% (P<0.01) after irradiation with oxygen ions, while the nuclear area of the dark epithelial cells increased with 6.82% (P<0.01). The cytoplasmic and cell area of both types of epithelial cells increased respectively with 8.86% and 6.62% (P<0.001) of the light cells and with 18.62% and 16.69% (P<0.001) of the dark cells after irradiation with oxygen ions. The results obtained after irradiation with fast neutrons were different. The nuclear, cytoplasmic and cell area of the light and dark epithelial cells diminished respectively with 16.86%, 6.71% and 7.10% (P<0.001) of the light cells and with 10.44%, 12.29% and 12.01% (P<0.001) of the dark cells in comparison with control. The irradiation with gamma rays did not provoke statistically significant differences in the morphometrical data of the light and dark epithelial cells. Statistically significant differences of the relative part were found after irradiation with oxygen ions (P<0.10) and gamma rays (P<0.02), when the relative part of the dark epithelial cells increased, while the relative part of the light cells decreased after the same irradiations.

The significant changes three months after fast neutrons irradiation were approximately 13% reduction (P<0.001) in the number of vessels of 5-7μm in diameter and 22% reduction (P<0.001) in vessels of 7-16μm in diameter. A significant increase of 32% was seen in the number of large vessels of 16-30μm (P<0.001) and 4% in vessels >30μm 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 5-7μm in diameter and 2% reduction in vessels of 7-16μm in diameter. A significant increase of 7% was seen in the number of large vessels of 16-30ìm (P<0.01) and >30μm (P<0.01) in diameter after irradiation with oxygen ions. Slightly different are the results obtained after exposure to gamma rays, when approximately 10% reduction (P<0.001) in the number of vessels of 5-7μm and 16-30ìm in diameter was seen (Fig. 5). Significant changes were not seen in luminal diameter and luminal area of all blood vessels after low doses irradiations.

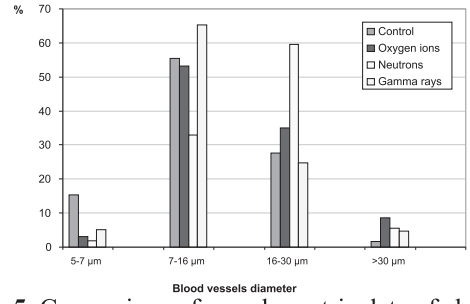


**Fig. 5.** Comparison of morphometric data of choroid plexus blood vessels of control rats and oxygen ions, neutrons and gamma rays irradiated rats (% -relative part)

## Discussion

The total-body exposures to low doses fast neutrons and gamma rays provoked similar ultrastructural alterations in the rat choroid plexus. Most of the epithelial cells exhibited ultrastructural signs of increased transcellular transport of substances. Similar ultrastructural changes of the rat choroid plexus epithelium were seen after irradiation with high energy carbon ions [6]. This increased intensity of the cellular transport relates, probably, with early post-exposure edema or subserves elimination of toxic substances consequent to radiation exposure [7]. Ultrastructural changes in the choroid plexus epithelial cells after exposure to high energy oxygen ions included increased absorption-secretion activities and stimulation of the cell metabolism that could be a compensatory reaction to the changes shortly after the experimental exposure [8].

The obtained morphometrical data of rat choroid plexus have shown that the nuclear and cytoplasmic area of both types of epithelial cells is changed statistically significant after irradiation with oxygen ions and neutrons. Statistically significant differences were found after irradiation with oxygen ions and gamma rays, when the relative part of the dark epithelial cells increased respectively by 8.2% and 12.7%. The obtained morphometrical data have shown that the changes of the choroid plexus epithelial cells are more marked after irradiation with oxygen ions and neutrons in comparison with gamma rays [9].

In the choroid plexus of rat brain, which has a relatively simple vascular system, significant changes were shown in the morphometrical data of blood vessels after irradiation with small doses of high energy oxygen ions, fast neutrons and gamma rays. 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, 56.34% in oxygen ions and 70.52% in gamma rays 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. These changes may be indicative of compensatory reactions in the organism following radiation exposure [10, 11]. Experimental study has 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 [12, 13].

## Conclusion

In conclusion, it can be pointed out that the applied irradiations provoked significant changes in rat choroid plexus epithelial cells and 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, oxygen ions and gamma rays. 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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