Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 22
Sofia ● 2015

Morphometrical Study of the Choroid Plexus Blood Vessels in Experimental Hamster *Graffi* Tumor Model

V. Ormandzhieva, R. Toshkova

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

The choroid plexus consists of epithelial cells, fenestrated blood vessels, and the stroma, dependent on various physiological or pathological conditions. In the present study the blood vessels, divided in four subgroups of the choroid plexus of control and tumor bearing hamsters (TBH), were morphometrically investigated. The investigations were performed on semithin sections examined with the light microscope using a square grid system. Brain tumor can be classified into two major classes, namely, primary brain tumor that start in the brain and secondary brain tumor that are generated by the cancer cells that migrated from tumor developed in other parts of the body. In the present study were observed statistically significant increase of the luminal diameter of the blood vessels in TBH on the 10^{th} and 30^{th} day of examination in comparison with control hamsters and metastasis near the brain ventricles. The morphological changes in the choroid plexus vasculature and structure are evidence for alteration of the blood-cerebrospinal fluid barrier and probably were a result of secondary metastasis in the brain.

Key words: choroid plexus morphological and morphometrical studies, experimental hamster *Graffi* tumor model, brain metastasis.

Introduction

Secondary brain tumors are more common than primary ones and are the most common cause of tumors in the intracranial cavity. This means that a cancerous neoplasm has developed in another organ elsewhere in the body and that cancer cells have leaked from that primary tumor and then entered the lymphatic system and blood vessels. They then circulate through the bloodstream, and are deposited in the brain. How the metastatic process is regulated also largely remains a mystery. The development of new therapeutic approaches for this disease is a difficult challenge, and there is no effective treatment for almost all the brain diseases. In most of the cases, the major cause of the failure in the development of drugs to treat brain diseases is the presence of blood-brain barrier (BBB) [1]. The cerebrospinal fluid (CSF) circulatory system is involved in the neuroimmune regulation, cerebral detoxification, and delivery of various endogenous and exogenous substances [2]. The barriers of the brain play critical roles in controlling the movement of various metabolites, but also drugs, between the blood and the brain (Blood-Brain Barrier) and the blood and the CSF (Blood-CSF-Barrier). Fundamental to all brain barrier mechanisms is the presence of intercellular tight junctions between

intimately opposed cells comprising these interfaces (endothelial cells of the brain vessels – BBB and choroid plexus epithelial cells – B-CSF-B) [8].

Plexus choroideus is highly vascularised structure in the brain ventricles. It produces cerebrospinal fluid and involves in the synthesis and transport of numerous CSF components. Choroid plexus has an important role in the homeostasis of nutrients in the CSF [13]. The transplantable myeloid tumor used in this study originated as a Graffi murine leukemia virus-induced tumor in newborn hamsters, adapted and maintained to mature Golden Syrian hamsters [3, 9].

The *aim* of the present study is to investigate the morphometric changes of the choroid plexus blood vessels in the experimental hamster *Graffi* tumor model.

Materials and Methods

Experimental hamster Graffi tumor model

Golden Syrian hamsters, 2 months old, were used in experiments. The experimental Graffi tumor was primary created by the Graffi-virus in new-born hamsters, and maintained monthly *in vivo* by subcutaneous transplantation of live tumor cells (2×10^6/ml PBS) in the interscapular area of hamsters, for keeping the tumor's survival [3, 9, 10]. The tumor is 100% cancerous, and the animals die usually up to the 30^{th} day after transplantation. The animals were kept under standard conditions with free access to food and water.

Histopathological examination

Brain samples from control (healthy) and tumor bearing hamsters (TBH) were taken, fixed in Carnoy's solution and embedded in paraffin using routine histological practice. Tissue sections (5–7 μm) were stained by hematoxylin-eosin and examined under light microscope Leica DM5000B.

Morphometric analysis

We obtained morphometric data from the light microscope Carl Zeiss Jena at 1000 × magnification using a square grid system. The luminal diameter was measured as perpendicular distance across the maximum chord axis of each vessels of control ($n = 446$) and TBH ($n = 431$ on the 10^{th} and $n = 467$ on the 30^{th} day after tumor implantation).

Statistical analysis: Results are reported as mean values ± SEM and statistically analyzed by Student's t-test using statistical package (STATISTICA, ver.6, Stat-Soft Inc., 2001), and differences were regarded as significant at $p < 0.05$.

All studies were performed in accordance to the Guide for Care and Use of Laboratory Animals, as proposed by the Committee on Care Laboratory Animal Resources, Commission on Life Sciences and National Research Council, and a work permit No. 11130006.

Results

In the present study changes in the luminal diameter of the choroid plexus blood vessels divided in four subgroups in control (healthy) and tumor-bearing hamsters were determined. These findings are shown in **Fig. 1.** Significant changes were observed in the luminal diameter of capillaries (vessels < 15.0 μm in diameter) and large vessels

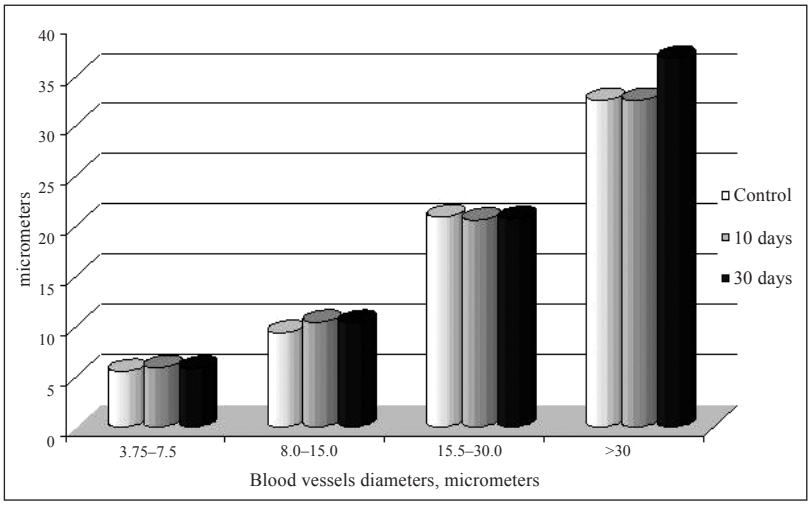

Fig. 1. Comparison of morphometric data of choroid plexus blood vessels in control and experimental hamster tumor model

(> 30.0 μm in diameter) in TBH on the 10th ($p < 0.001$) (blood vessels diameter of the 3.75-7.5 μm: 5.90 ± 0.10 μm and 8.0-15.0 μm: 10.42 ± 0.24 μm) and 30th ($p < 0 .001$) (blood vessels diameter of the 3.75-7.5 μm: 5.83 ± 0.11 μm and 8.0-15.0 μm: 10.40 ± 0.25 μm; blood vessels > 30.0 μm in diameter: 36.70 ± 1.23 μm) day of examination in comparison with control hamsters (blood vessels diameter of the 3.75-7.5 μm: 5.54 ± 0.09 μm and 8.0-15.0 μm: 9.38 ± 0.21 μm; blood vessels > 30.0 μm in diameter: 32.50 ± 0.91 μm). The mean luminal diameter of capillaries (blood vessels diameter of the 3.75-15.0 μm) was not statistically changed in TBH in comparison with control.

The choroid plexus lies in the brain ventricles as a fibrous network of tissue and vasculature. It consists of single layer of large cuboidal light and dark epithelial cells, connective tissue elements and capillary with many fenestrations (**Fig. 2**). The histopathological studies of the brain on the 10th and the 30th day after tumor implantation are shown in **Figs. 3** and **4**. Many brain vessels and choroid plexus cappilaries with destructive changes were seen in the present TBH study. Some endothelial cells of the choroid plexus blood vessels were destroyed. There were many dark epithelial cells and macrophages in the apical part of the choroid plexus epithelial cells. Massive accumulation of tumor metastatic cells were detected in the brain tissue,

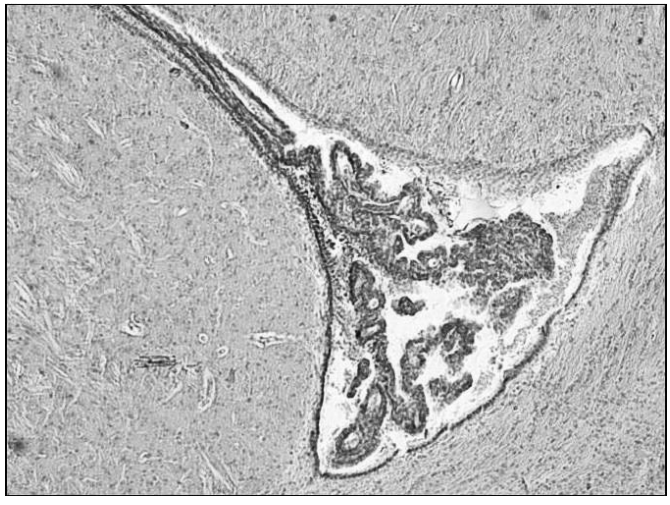

Fig. 2. Light microscopic micrograph of the plexus choroideus of control hamster in the lateral ventricle. H&E stain (× 5)

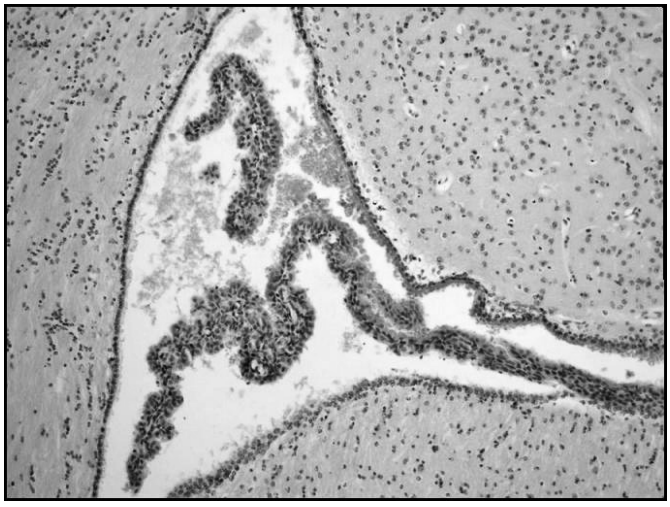

Fig. 3. Light microscopic micrograph of the plexus choroideus of tumorbearing hamster (10 days after tumor implantation). H&E stain (× 10)

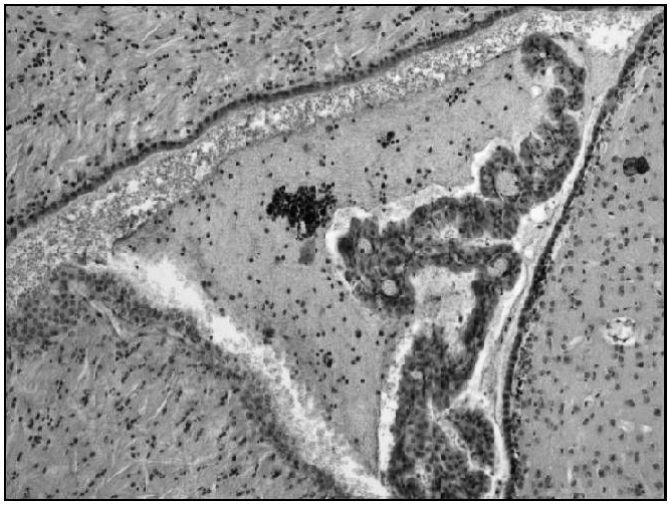

Fig. 4. Light microscopic micrograph of the plexus choroideus of tumorbearing hamster (30 days after tumor implantation). H&E stain (× 10)

lateral ventricle and under ependyma in TBH on the 30th day of examination. Tumor cells have a similar morphological characteristic to that described in the primary tumor.

Discussion

For the first time brain was examined in experimental hamsters with Graffi tumor to establish morphological changes in the choroid plexus and brain metastases during tumor progression. Up to present time little quantitative information has been available

regarding the vessels of the brain, in particular, regarding the vessels of the rat choroid plexus. The capillaries of the choroid plexus have a large diameter and sinusoidal dilations, and showed the presence of occasional short, blind sprouts indicative of angiogenesis. Short anastomoses between arterioles supplying the plexuses and venules draining them were only rarely observed [12].

It was found that the tumor metastasizes lymphatic and haematogenous route. In our previous studies metastases were observed in regional and non-regional lymph nodes, in the lung, liver and spleen of TBH. Massive accumulation of tumor metastatic cells were detected in the brain tissue, lateral ventricle and under ependyma in TBH on the 30th day of examination. Tumor cells in metastatic lesions have a similar morphological characteristic to that described in the primary tumor, reported in our previous studies [9, 11].

Remarkable changes were found in choroid plexus morphology in all tumorbearing hamsters. The significant increases were observed in the luminal diameter of capillaries and large vessels in TBH on the 10th (vessels diameter of the 3.75-7.5 μm by 6.50% and 8.0-15.0 μm by 11.09%) and 30th (vessels diameter of the 3.75-7.5 μm by 5.23% and 8.0-15.0 μm by 10.87%; blood vessels > 30.0 μm in diameter by 12.92%) day of examination in comparison with control hamsters. Destructive changes of the brain vessels and choroid plexus capillaries, many macrophages in the apical part of the choroid plexus epithelial cells and in the CSF, and increased number of dark epithelial cells, were seen in the present study. Most blood vessels in the plexus choroideus are wide-calibre (10-15 μm) fenestrated capillaries, wich included non-fenestrated endothelial segments with some vesicles [4]. Similar changes in the rat choroid plexus blood vessels and epithelial cells we observed in our previous investigations after low doses ionizing irradiation [5, 6]. Statistically significant changes in the choroid plexus blood vessels established in this study are in support of our previous research in which we found metastatic lesions in the brain ventricles and brain tissue [7]. The choroid plexus epithelium constitutes a physical barrier between blood and cerebrospinal fluid (the blood – CSF barrier – BCSFB) by virtue of the complexity of the tight junctions between adjacent epithelial cells.

Conclusion

Our morphological study of the TBH experimental model clearly demonstrated that the brain metastasis provoked significant destructive changes in the plexus choroideus. These morphological changes led to the damage of the BBB and B-CSF-B.

Acknowledgements: This research was financially supported by the Bulgarian National Science Fund (Grant BO2/5-2014). The authors wish to thank M. Pavlova, medical laboratory assistant, for the excellent technical assistance of the histological preparation of tissue.

References

1. **Bhowmik A., R. Khan, M. K. Ghosh.** Blood brain barrier: A challenge for effectual therapy of brain tumors. – BioMed. Research International, 2015, (http://dx.doi.org/10.1155/2015/320941).
2. **Ghersi-Egea, J., A. Babikian, S. Blondel, N. Strazielle.** Changes in the cerebrospinal fluid circulatrory system of the developing rat: quantitative volumetric analysis and effect on blood-CSF permeability interpretation. – Fluids and Barriers of the CNS, **12**(8), 2015, DOI 10.1 186/s12987-015-0001-2.

3. **Jakimov, M., Z. Mladenov, A. Konstantinov, I. Yanchev.** Transplantable myeloid tumor in hamsters (MTH) induced by Graffi virus. – Gen. Compar. Pathol., **6**, 1979, 24-35.
4. **Ormandzhieva, V.** Rat choroid plexus blood vessels: morphometric characteristics. – Med. Data Rev., **3**(3), 2011, 229-232.
5. **Ormandzhieva, V.** Effect of radiation with low doses fast neutrons and high energy oxygen ions on the rat choroid plexus blood vessels. – Acta Morphol. Anthropol., **13**, 2008, 22-25.
6. **Ormandzhieva, V.** Choroid Plexus after Low Doses Ionizing Irradiation. – Med. Data Rev., **5**(2), 2013, 111-119.
7. **Ormandzhieva, V., R. Toshkova.** Brain metastasis in *Graffi* hamster experimental model. – Proceedings of the Fifth workshop experimental models and methods in biomedical research, 2015, 12-14 May, (http://www.iempam.bas.bg/).
8. **Saunders, N., R. Daneman, K. Dziegielewska, S. Liddeow.** Transporters of the blood-brain and blood-CSF interfaces in development and in the adult. – Molecular Aspects of Medicine, **34**, 2013, 742-752.
9. **Toshkova, R.** Attempts for immunomodulation in hamsters with transplanted myeloid tumor, previously induced by Graffi virus. PhD Thesis, 1995, Sofia, Bulgaria, p.168.
10. **Toshkova, R., N. Manolova, E. Gardeva, M. Ignatova, L. Yossifova, I. Rashkov, M. Alexandrov.** Antitumor activity of quaternized chitosan-based electrospun implants against Graffi myeloid tumor. – J. Intern. Pharm., **400**(1-2), 2010, 221-233.
11. **Yanchev, I., R.Toshkova, E. Ivanova.** Immunomorphological changes in hamsters with progressing myeloid tumor induced by Graffi virus. – Comptes rendus de l'Academie bulgare des Sciences, **49**(3), 1996, 95-98.
12. **Zagorska-Swiezy K., J. Litwin, J. Gorczyca, K. Pitynski, A. Miodonski.** The microvascular architecture of the choroid plexus in fetal human brain lateral ventricle: a scanning electron microscopy study of corrosion casts. – J. Anatomy, **213**(3), 2008, 259-265.
13. **Орманджиева, В**. Електронномикроскопски и хистометрични изследвания на plexus choroideus в мозъка на плъх в онтогенеза и при експериментални въздействия. – София, АИ „Проф. М. Дринов", 2014. 152 с.

Corresponding author:
e-mail: vormandzhieva@abv.bg