

ULTRASTRUCTURAL STUDY OF THE LATERAL VENTRICLE CHOROID PLEXUS IN EXPERIMENTAL HYDROCEPHALUS IN WISTAR RATS

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ABSTRACT - Hydrocephalus is one of the most frequent and complex neurological diseases characterized by the abnormal buildup of cerebrospinal fluid (CSF) in the ventricles of the brain, due to an altered CSF dynamics. To detect possible ultrastructural alterations of the lateral ventricles choroid plexus (responsible for the CSF production), rats seven days after birth were submitted to an intracisternal injection of 20% kaolin (hydrated aluminum silicate) for the hydrocephalus induction. Twenty-eight or 35 days after injection, injected animals and respective controls were processed for observation under a transmission electron microscopy. Alterations found: presence of concentric cell membrane fragments, larger number of primary and secondary lysosomes, vacuoles, and cytoplasmic vesicles, and an enlargement of the intercellular space and between the basolateral interdigitation of the choroid epithelium. The alterations observed are probably associated to an increase of the ventricular pressure, inducing morpho-functional effects on the choroid plexus integrity.

KEY WORDS: hydrocephalus, choroid plexus, ultrastructural study, rat.

Estudo ultraestrutural dos plexos corioides dos ventrículos laterais de ratos Wistar submetidos a hidrocefalia experimental

RESUMO - A hidrocefalia é uma das mais frequentes e complexas doenças neurológicas caracterizada pelo acúmulo de líquido cefalorraquidiano (LCR) no interior dos ventrículos cerebrais e conseqüente alteração na dinâmica liquórica. Para detectar as possíveis alterações ultra-estruturais nos plexos corioides dos ventrículos laterais (responsáveis pela produção do LCR), ratos sete dias após o nascimento, foram submetidos à indução de hidrocefalia pela injeção intracisternal de caulim a 20%. Após 28 e 35 dias da injeção, estes animais e seus respectivos controles foram processados para observação em um microscópio eletrônico de transmissão. Alterações observadas: presença de membranas concêntricas, maior número de lisossomos primários e secundários, vacúolos e vesículas citoplasmáticas, aumento do espaço intercelular e entre as interdigitações basolaterais das células do epitélio coriódio. As alterações observadas possivelmente estão associadas ao aumento da pressão nos ventrículos, induzindo efeitos morfo-funcionais na integridade dos plexos corioides.

PALAVRAS-CHAVE: hidrocefalia, plexos corioides, estudo ultra-estrutural, rato.

Hydrocephalus is one of the most frequent and complex neurological diseases characterized by the abnormal buildup of cerebrospinal fluid (CSF) in the ventricles of the brain, due to an altered CSF dynamics. The hydrocephalus is a result of multiple pathophysiological mechanisms, with many different causes including a birth defect, hemorrhage into the brain, infection, meningitis, tumor, or head injury. In this way, the treatment possibilities as also the ideal time to introduce it has been widely studied¹. Kaolin has been widely used to induce hydroceph-

alus in several developmental stages of experimental animals including neonates and adults. This model of experimental induction was chosen due to it being efficient, inexpensive, and it does not need surgical proceedings or cause anatomic modifications and does not even lead to other alterations unless those results that are totally of hydrocephalus²⁻⁷.

As responsible for the CSF production, the choroid plexus play an important role on the central nervous system (CNS) homeostasis. Because of its situation in the lateral ventricles of the brain, the choroid plexus

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are involved by hydrocephalus in its early stages^{8,9}. Del Bigio et al.¹⁰ and Kiefer et al.¹¹ observed reduced density of ependymal microvilli, vacuolar formation and cellular inclusions on choroid cells, and intercellular space enlargement, after experimentally induced hydrocephalus. Go et al.¹² and Madhavi and Jacob¹³ observed the presence of Kolmer fagocytic cells on the ependymal cells surface and on the choroid cells in hydrocephalic animals. Also, by means of morphometry, these authors have found a significant decrease of surface volume and area of the choroid epithelium in hydrocephalic animals, compared to controls. Alterations on the choroid plexus in hydrocephalic animals are not well established in the literature.

In order to contribute for a better understanding of the hydrocephalus pathologic alterations of brain structures, the aims of the present study were to detect, by means of transmission electron microscopy (TEM), the alterations on lateral ventricles choroid plexus of Wistar rats, with experimentally induced hydrocephalus.

METHOD

Twenty-four litters of 7-day old Wistar rats were used, without sexual distinction. Each litter was constituted of the mother rat and 8 newborn rats. Two groups of animals were submitted to the hydrocephalus induction with intracster-

nal injection of 20% kaolin (after 28 or 35 days of injection) and the third group was used as control^{14,15}. Each pup rat was held by an auxiliary, who held the head with one hand and the body with the other, bending the animal's neck, leaving the posterior cervical area free. By palpation the space between the posterior extremity of the foramen magnum, on the occipital bone, and the posterior arch of the first cervical vertebra was identified. With a Mise 0.3 odontologic needle, with a short bezel, the suboccipital percutaneous injection was done, and 0.4 mL of a kaolin suspension (Merck) diluted in distilled water of 20% was slowly injected. Following this the rats were put back into their cages with their mothers.

The animals of each group injected with kaolin, and their respective controls of the same age, were anesthetized with vapors of sulphuric ether and submitted to a transcardiac perfusion with PBS solution (about 1 mL/g of the animal's weight), after the perfusion, with the same volume, with a 2% paraformaldehyde and 1% glutaraldehyde in a 0.1M, pH 7.4 phosphate buffer. The animals were decapitated, their brains removed in block by a craniectomy of the vertex and immersed in the same fixative solution of formaldehyde and glutaraldehyde for more than 24 hours at 4°C.

After washing with a 0.1M phosphate buffer, the brains were sectioned at the coronal plane, and the choroid plexuses identified and removed. The samples from the choroid plexuses were fixed in a 1% osmium tetroxide in a 0.2M phosphate buffer for 2 hours, at 4°C, washed with a 0.1M phosphate buffer and then dehydrated in an increasing series of acetone and embedded in araldite. After the polymerization of the resin, the blocks were cut in sections of 0.5 µm (semi-

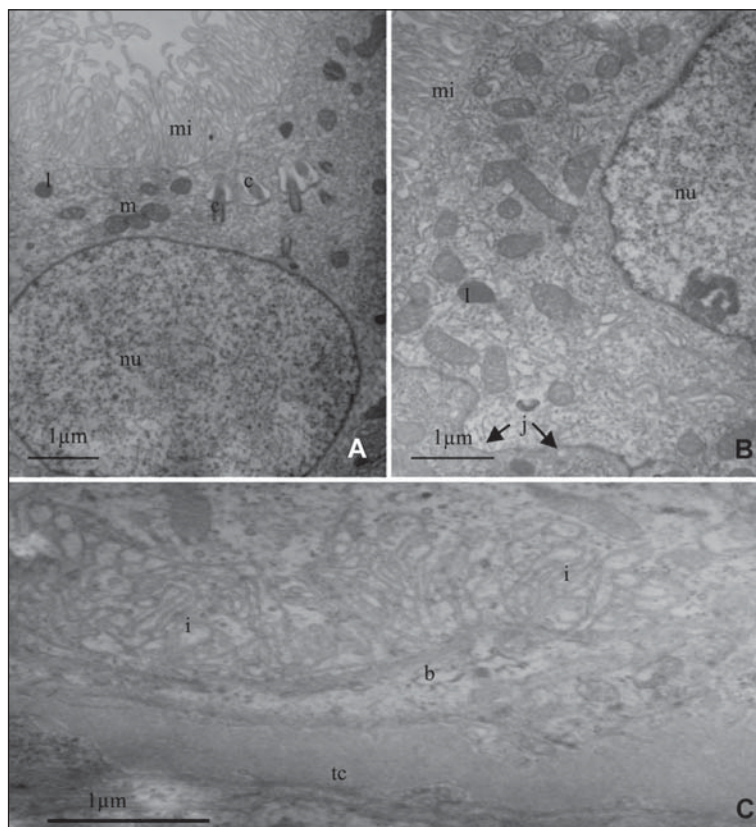


Fig 1A, 1B and 1C. Electron micrographs of the lateral ventricle choroid plexus of control rats. Cilia (c); interdigitation (i); intercellular junction (j); basal lamina (b), lysosome (l); microvilli (mi), mitochondria (m); nucleus (nu), connective tissue (tc). 10000x; 13000x, 13000x.

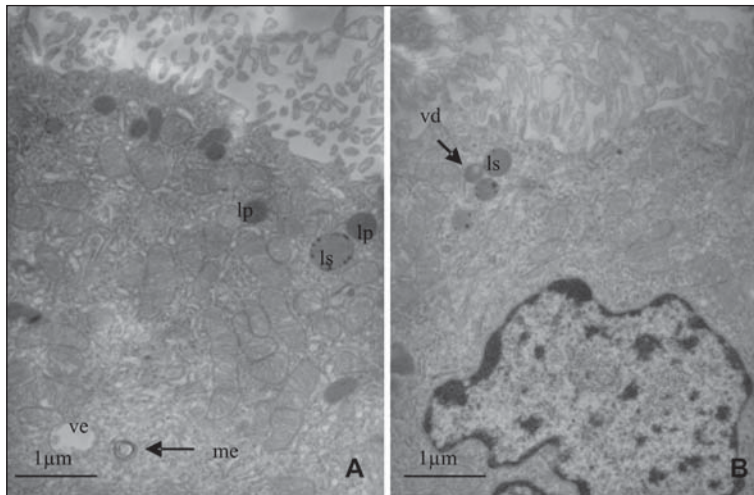


Fig 2A and 2B. Electron micrographs of the lateral ventricle choroid plexus of rats 28 (2A) and 35 (2B) days after hydrocephaly induction. Concentric membranes, similar to myelin (me); primary (lp) and secondary (ls) lysosomes; digestive vacuoles (vd), electron-lucid containing vesicles (ve). 13000x; 11000x.

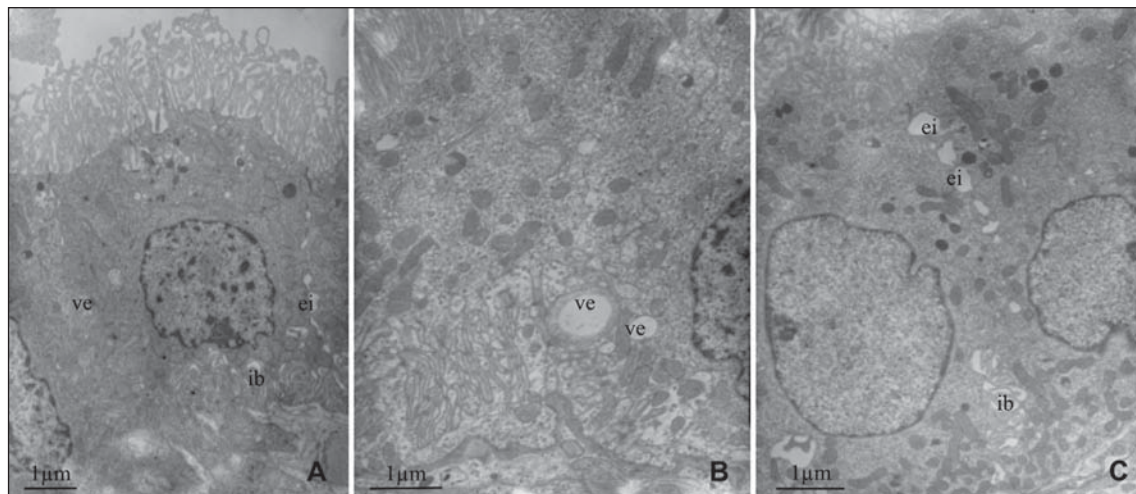


Fig 3A, 3B and 3C. Electron micrographs of the lateral ventricle choroid plexus of rats 35 days after hydrocephaly induction. Enlargement of the intercellular space (ei) and the basolateral interdigitation (ib); electron-lucid containing vesicles (ve). 8000x; 13000x; 10000x.

fines) and after 500Å of thickness. The sections were stained with uranyl and lead citrate and examined in a EM 208 Philips TEM. All experimental procedures were performed accordingly with the Ethical Principles in Animal Research adopted by Brazilian College of Animal Experimentation (COBEA) and was approved by the College of Medicine of Ribeirão Preto of the University of São Paulo – Ethical Commission of Ethics in Animal Research (CETEA), protocol n° 133/2006.

RESULTS

The choroid epithelium is ultrastructurally described as simple columnar, presenting many microvilli on the ventricle surface and the occasional presence of cilia¹⁶ (Figs 1A and 1B). The cells cytoplasm presents a large central nucleus, mitochondria, rough endoplasmic reticulum, Golgi complex and occasional lysosomes. A joint complex is found on the apical junction of the cells (Fig 1B) as a basolateral membrane

full of interdigitation (Fig 1C). Choroid epithelial cells lay on a dense basal lamina. Beneath this lamina, a small amount of loose connective tissue with collagen fibers separate the basal lamina (Fig 1C) and the main capillary vessels, formed by a thin endothelium¹⁷⁻²¹.

On animals of the control groups, no alterations were seen. On both experimental groups (28 and 35 days after kaolin injection), the following alterations were found: presence of concentric cell membrane fragments, similar to the myelin sheath on the cytoplasm (Fig 2A), larger number of primary and secondary lysosomes (Figs 2A and 2B), vacuoles (Fig 2B), and cytoplasmic vesicles of different sizes, containing an electron-lucid material (Figs 2A, 3A and 3B). On animals 35 days after kaolin injection, an enlargement of the intercellular space and between the basolateral interdigitation of the choroid epithelium was also

seen (Figs 3A and 3C).

DISCUSSION

Among the main functions of the choroid plexus is the CSF production. Nevertheless, it is well known the role of the choroid plexus in the nutrition and protection of the CNS since the CSF is a chemically stable fluid^{19,21}. The choroid plexus is able to transfer nutrients to the CSF, actively regulating the molecules concentration of this fluid and determining the selective characteristics of the blood-CSF barrier²¹. The choroid plexus also present a role on the CSF clearance of drugs and substances formed on the brain tissue due to the metabolic reactions, thus presenting several active-transport systems^{9,21}. The first structure to suffer on the hydrocephalus process in high CSF pressure is the ependymal epithelium, with compression, distension and isolated disruption^{10,11}.

Go et al.¹² and Go and Molenaar²² observed the infestation of Kolmer fagocytic cells on the ependymal cells and choroid cells surface, as well as a degeneration of ependymal cilia. The fragments observed on the choroid plexus surface of hydrocephalic animals are consequence of an impaired ciliar movement due to the ciliar degeneration described previously^{12,22}. In this way, an altered CSF dynamics and the CSF accumulation on an enlarged ventricular system impairs the correct elimination of the metabolic products²³.

Del Bigio et al.¹⁰, Madhavi and Jacob¹³ and Kiefer et al.¹¹ have observed alterations on the choroid epithelium such as microvilli distortion, atypical cilia, flatten cells with and vacuoles, intracellular inclusions and enlargement of intercellular space. Some of these alterations were also seen on the present study. In this way, the vesicles containing electron-lucid material observed on the choroid cells might be the result of a fluid penetration into the cytoplasm. The presence of a large amount of lysosomes, secondary lysosomes and digestive vesicles might suggest that there is a secretion and absorption impairment of CSF products or the cytoplasmic organelles death. In a morphometric study, Madhavi and Jacob^{24,25} have shown a significant decrease on the volume and area of the choroid epithelium in hydrocephalic animals. Also, these authors showed a reduction of the mitochondrial internal membrane area, including the crests, which was attributed to a decreased activity of the choroid cells, followed by a reduction on the CSF secretion on those animals.

In conclusion, the choroid plexus is the main structure responsible for production and maintenance of

the CSF homeostasis, an essential factor for the development and normal functioning of the CNS. In this way, the alterations observed in this structure are associated to an increase of the ventricular pressure, inducing morpho-functional effects on the choroid plexus integrity, contributing to several morpho-functional alterations of the CNS²⁶.

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