

Case Report

A choroid plexus cyst in the fourth ventricle of a Sprague-Dawley rat

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Abstract: Choroid plexus cysts are rare lesions in the brain and are reported in humans and dogs. Herein, we report a choroid plexus cyst found in a 10-week-old female Sprague-Dawley rat. Histologically, a cyst measuring approximately 600 μm in diameter was found in the fourth ventricle of the brain. The cyst was lined with a single layer of flattened cells and was present in the connective tissue of the choroid plexus. Next to the cyst, a dilated tube was found with a similar morphology to the epithelium of the choroid plexus. Immunohistochemistry revealed that flattened cells lining the cyst were positive for cytokeratin and vimentin, and negative for GFAP and S-100, which is the same as in the normal choroid plexus, excluding vimentin. We diagnosed the present cyst as a spontaneously occurring choroid plexus cyst that was considered to be undergoing the epithelial-mesenchymal transition. (DOI: 10.1293/tox.2017-0012; *J Toxicol Pathol* 2017; 30: 235–238)

Key words: choroid plexus cyst, rat, fourth ventricle, cytokeratin, vimentin

Choroid plexus cysts (CPCs), small fluid-filled structures in the brain, have been reported in humans and dogs. In humans, CPCs are generally found in the lateral ventricle^{1, 2} and are detected in 1–3.6% of all fetuses in routine mid-gestation ultrasounds³. Of the CPCs detected in fetuses, 90% regress during development and are considered to be of no clinical significance³. On the other hand, CPCs are found in 11% of children 10 years old and under and are found more frequently in older people in routine postmortem examinations². These cysts are generally less than 1 cm in diameter and do not cause obstructive symptoms². Symptomatic cysts are usually larger and cause symptoms such as episodic headaches or seizures due to increased cranial pressure^{1, 2}. In dogs, CPCs are rare findings and have been reported in two adult animals^{4, 5}. In one dog, a CPC was found in the fourth ventricle and was surgically removed, thereby resulting in a good prognosis⁴. The other dog had a cyst in the medulla oblongata, which made it difficult to resect it, and this dog was euthanized due to progressive and severe clinical signs⁵. Meanwhile, choroid plexus tumors have been reported as spontaneously occurring lesions in rats^{6, 7}, and vacuolations in the choroid plexus are known as a drug-induced pathological change⁸. However, there are no

reports of CPCs in rats. Herein, we report histopathological and immunohistochemical characteristics of a CPC found in a rat.

Sprague-Dawley (CrI:CD(SD)) rats were obtained from Charles River Laboratories Japan, Inc. (Kanagawa, Japan) at 4 weeks of age and were used in a 4-week toxicity study. Based on national regulations and guidelines, the animal experiments were reviewed by the Institutional Animal Care and Use Committee of our research institution and ultimately approved by its director. The animal experiments were performed in accordance with Regulations for Animal Experiments of ONO Pharmaceutical Co., Ltd. All rats were individually housed in stainless steel wire cages in the animal rooms maintained at a temperature of $23 \pm 2^\circ\text{C}$ and a humidity of $55 \pm 10\%$ with a 12-hour light/dark cycle. The rats had access to a commercial diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and ultrafiltered drinking water. The female rat described in this case report was allocated to the high dose group and orally received the test article for 4 weeks. During the administration period, the rat did not exhibit any abnormalities in general condition, food consumption, or body weight gain. After the 4-week administration, the rat, which was 10 weeks old, was euthanized for necropsy by exsanguination from the abdominal aorta under inhalation anesthesia with isoflurane. At necropsy, no abnormalities were found in the body, including the brain, or in organ weight. Major organs were excised and fixed in 10% phosphate-buffered formalin, routinely embedded in paraffin, and sliced into 2- to 3- μm -thick sections. The sections were stained with hematoxylin and eosin (HE), and serial sections were used for immunohistochemical staining with the primary antibodies listed in Table 1. Briefly,

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Table 1. Primary Antibodies for Immunohistochemistry

Antibody	Supplier	Host	Dilution	Antigen retrieval
Cytokeratin (MNF116)	OriGene Technologies, Rockville, USA	Mouse	1:100	Proteinase K for 5 min at room temperature
Vimentin (V9)	Dako, Tokyo, Japan	Mouse	-	Autoclaving at 125°C for 30 sec
GFAP (GA5)	Cell Signaling Technology, Tokyo, Japan	Mouse	1:800	Autoclaving at 125°C for 30 sec
S-100	Dako, Tokyo, Japan	Rabbit	1:1600	-

-, not done

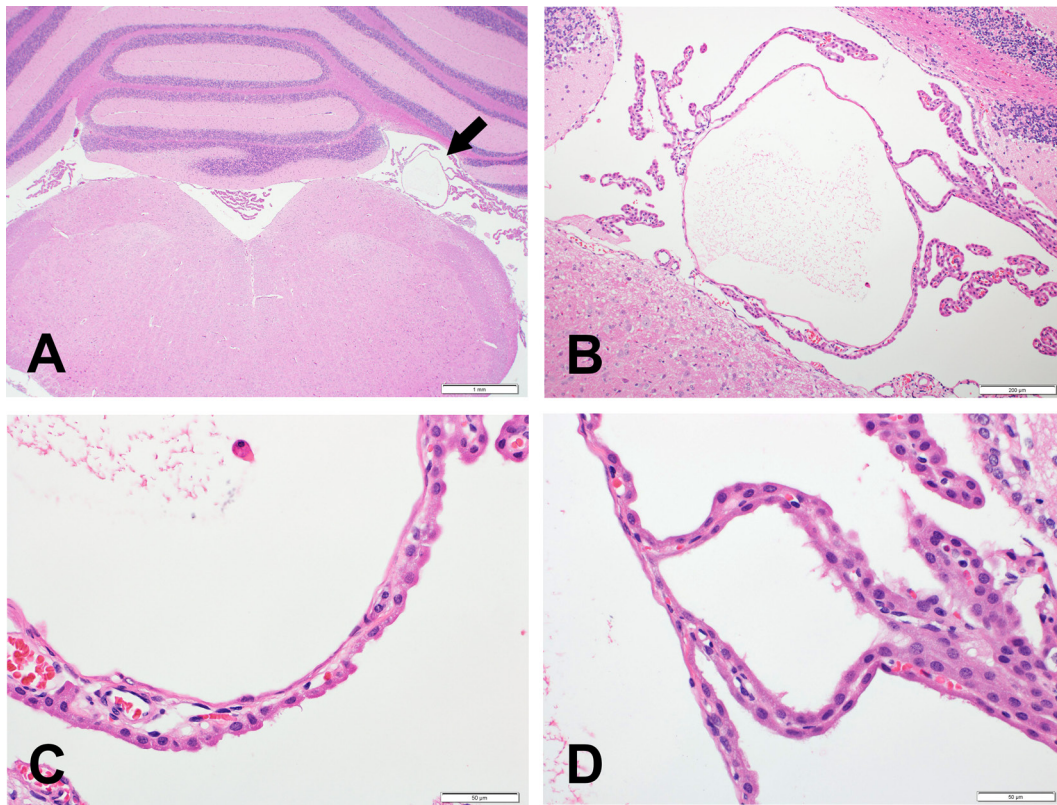


Fig. 1. A choroid plexus cyst in the brain. HE stain. A) The cyst (arrow) was located in the fourth ventricle of the brain. B) The cyst was connected with the normal choroid plexus. C) A single layer of flattened cells lined the cyst. D) Dilation of a tube was found beside the cyst. Bars = 1 mm (A), 200 μ m (B), and 50 μ m (C and D).

the sections were incubated with a primary antibody at 4°C overnight. After washing and incubation with a peroxidase-labeled secondary antibody, the sections were visualized with 3,3'-diaminobenzidine tetrahydrochloride, followed by counterstaining with hematoxylin.

Histologically, a cyst measuring approximately 600 μ m in diameter was found in the fourth ventricle of the brain (Fig. 1A). The cyst was connected with a normal choroid plexus and separated from the brain parenchyma (Fig. 1B). The cyst wall was lined with a single layer of flattened cells that showed indistinct cell borders. Interestingly, the cyst was present in a connective tissue stroma of the choroid plexus, where the cyst wall was covered by two layers consisting of scant connective tissue stroma containing blood vessels on the inside and the epithelial cells of the choroid plexus on the outside. The cyst's cavity contained a few eosinophilic materials (Fig. 1C). Next to the cyst, dilation

of a tube was also present in the connective tissue stroma. The epithelial morphology of the tube was mostly cuboidal like that of the choroid plexus and was focally flattened like that of the cyst (Fig. 1D). Except for the cyst and tube, no other lesion was detected in the brain or other organs of this animal. Because no lesions were observed in the brains of the other dosed animals, we consider the present cyst to be a spontaneously occurring lesion. Immunohistochemistry revealed that a single layer of flattened cells of the cyst was positive for cytokeratin and vimentin, and negative for GFAP and S-100 (Fig. 2). Table 2 summarizes the results of immunohistochemical staining of the epithelial cells in the cyst, choroid plexus, and ependymal cells. The results for the choroid plexus and ependymal cells corresponded to those of previous studies^{7,9}. Unfortunately, we could not find the tube in the immunohistochemical specimens because it disappeared during slicing. The immunohistochemical

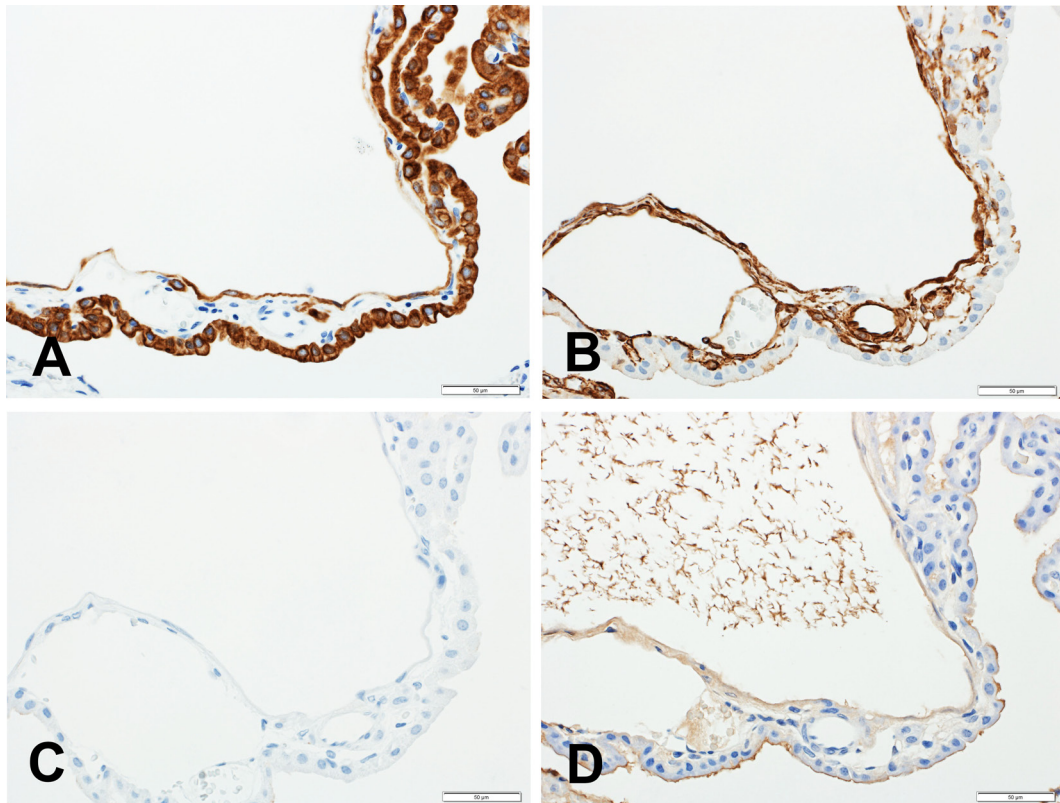


Fig. 2. Immunohistochemical staining of the choroid plexus cyst. The cyst wall was positive for A) cytokeratin and B) vimentin, and negative for C) GFAP and D) S-100. Bars = 50 µm.

Table 2. Immunohistochemical Results of the Cyst, Choroid Plexus, and Ependymal Cells

Antibody	Cyst	Choroid plexus	Ependymal cells
Cytokeratin (MNF116)	++	++	++
Vimentin (V9)	++	–	++
GFAP (GA5)	–	–	+
S-100	–	–	++

–, negative; +, focally positive; ++, mostly positive

characteristics of the present cyst corresponded to those of the choroid plexus, excluding vimentin. Considering its location, we diagnosed the present cyst as a CPC.

The choroid plexus is formed during embryonic development by invagination of a bilayer membrane that consists of the neuroectoderm and pia mater into the ventricular spaces¹⁰. During this period of growth of choroidal villi, CPCs are considered to be formed as follows: The neuroepithelium folds into the choroidal matrix and pinches off the folding neck, leading sacs to separate from the ventricle¹. As cerebrospinal fluid is secreted by the choroid plexus, cysts grow by fluid accumulation¹. While epithelial cells in the choroid plexus generally cover a scant connective tissue stroma⁸, those in the present cyst and tube were located in a connective tissue stroma. Because the dilated tube focally showed a flattened morphology similar to that of the cyst,

both lesions probably originated from the same cells, and the differences between them likely resulted from fluid accumulation. Therefore, the present cyst and tube are considered to have been formed by neuroepithelial folding into the connective tissue matrix during choroid plexus development, similar to that in humans.

CPCs have been reported in humans and dogs. Most CPCs have been reported in humans, probably because they can be detected in fetuses as temporary findings that resolve by the 26–28th week of gestation³. Because they are so small, 5 mm to 15 mm in diameter under sonographic examinations even in humans³, it may be difficult to detect cysts in animals using sonography even if they are present during the fetal period. Histologically, the cyst epithelia have been variously reported as flattened, low columnar, or cuboidal cells^{2,4,5}. These differences may be the result of the cyst's size as observed in the present case. Immunohistochemical characteristics of CPCs and the choroid plexus are diverse among species. In rats, the choroid plexus is positive for cytokeratin and negative for vimentin, S-100, and GFAP^{7,9}, which is similar to the findings in guinea pigs, excluding S-100, which was not tested⁹. Unlike rats, dogs show no expression of cytokeratin, vimentin, S-100, or GFAP in the choroid plexus or CPC⁵. In humans, the choroid plexus is positive for vimentin, cytokeratin, and S-100 but negative for GFAP¹¹. Some cysts express GFAP in addition to cytokeratin, suggesting that the choroid plexus could differenti-

ate into glial or ependymal cells¹². In rats, focal expression of GFAP is one of the immunohistochemical characteristics of ependymal cells^{7, 9}, but it was not found in the present cyst.

The epithelial cells in the present cyst showed positive reactivity with both cytokeratin and vimentin, whereas the normal choroid plexus shows positive reactivity only with cytokeratin in rats^{7, 9}. Similarly, the expression of vimentin in cyst epithelia has been reported in the kidney¹³ and the pituitary gland¹⁴. In polycystic kidney (PCK) rats, an orthologous model of human polycystic kidney disease, renal cysts undergo epithelial-mesenchymal transition (EMT) as they get larger, with epithelial cells in the cysts losing epithelial features and acquiring mesenchymal ones, such as expressions of vimentin and fibronectin¹³. EMT also contributes to the progression of renal failure in human polycystic kidney disease¹⁵. Being damaged chronically, epithelial cells are considered to undergo EMT so that they will not undergo apoptosis¹⁶. In the present case, the cyst epithelium was flattened probably due to chronic compression by fluid accumulation. Because coexpression of cytokeratin and vimentin is observed in the intermediate stage of EMT¹⁷, the cyst epithelium is considered to be undergoing EMT. EMT in renal cysts resembles that of the previous cell phenotype, where renal tubules coexpress cytokeratin and vimentin during development¹¹. The fact that the choroid plexus also expresses vimentin in rats during development¹⁸ supports our hypothesis of EMT in the CPC.

In conclusion, the present report described a CPC in the fourth ventricle in a rat. As it was located in the connective tissue of the choroid plexus, it may have been formed by neuroepithelial folding into the connective tissue stroma during choroid plexus development. Coexpression of cytokeratin and vimentin suggested that the epithelial cells of the cyst underwent EMT. To the best of our knowledge, this is the first report of a CPC in rats.

Disclosure of Potential Conflicts of Interest: The authors declare that there are no conflicts of interest associated with this paper.

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