Porencephaly with an optic organ abnormality in a beagle dog

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Short running head: Porencephaly with an optic organ abnormality in a dog

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Abstract

A female TOYO beagle dog showed porencephaly and visual organ abnormalities. At necropsy, there was a cavity filled with cerebrospinal fluid in the right cerebral hemisphere and an adhesion area between the cerebral cortex and the skull, which was partially thickened. Additionally, the right optic nerve showed a slight decrease in diameter. Histopathological examination revealed increased glial fibers and collagen fibers, hemosiderin deposition, and an increased number of microglia in the adhesion area, along with a marked reduction of the cerebral parenchyma. In the right eyeball, the retina and optic nerve showed focal atrophy in the nerve fiber layer and inner granular layer to full retinal atrophy and hypoplasia of the myelinated nerve fibers, respectively. Electron microscopic examination revealed hypoplasia of the myelin sheath of nerve fibers in the right optic nerve. This is an extremely rare case of porencephaly and congenital optic nerve hypoplasia, along with independent retinal thinning.

Keywords: porencephaly, TOYO beagle, optic nerve hypoplasia
Porencephaly is believed to be caused by fetal or postnatal infections, circulatory disorders, genetic anomalies, and trauma. In laboratory animals, there have been some reports on the occurrence of porencephaly in cynomolgus monkeys, but no such reports have been made in beagle dogs. In this study, we report the case of a beagle dog with porencephaly and visual organ abnormalities.

This case study included 12 male and 12 female 5-month-old TOYO beagle dogs that were purchased from Kitayama Labes Co., Ltd. (Yamaguchi, Japan) in 2015. The dogs were housed in pairs in a metal cage in an environmentally controlled animal room (temperature: 22 ± 4°C; relative humidity: 55 ± 25%; air ventilation: 13–15 times/h; and lighting: 12 h/day). This dog was given a 300 g pelleted diet (DS-A, Oriental Yeast Co., Ltd. Tokyo, Japan) daily and allowed free access to tap water through an automatic water supply system. The dog was handled according to the “Law Concerning the Protection and Control of Animals” (Japan Law No. 105, October 1, 1973), “Standards Relating to the Care and Keeping and Reducing Pain of Laboratory Animals” (Notice No. 88 of the Ministry of the Environment, 2006), and “Guideline for Animal Experimentation” (the Science Council of Japan, June 1, 2006) in Japan. This study was approved by the Animal Welfare Committee of the Bozo Research Center Inc. (Tokyo, Japan). Ophthalmological examination was performed at the age of six months after a month of quarantine and acclimatization and subjected to macroscopic, anterior eye, and the fundus
examinations of the eyes. Macroscopic observation included the exterior appearance and pupillary reflex using a penlight. In the anterior and fundus examinations, after the instillation of a mydriatic agent (tropicamide and 0.5% phenylephrine hydrochloride, Mydrin P, Santen Pharmaceutical Co., Ltd., Osaka, Japan) in both eyes, the cornea, conjunctiva, lens, iris, vitreous body, and fundus were examined using a slit-lamp microscope (SL-15, Kowa Co., Ltd. Tokyo, Japan) and a binocular indirect ophthalmoscope (Omega 500, Heine Optotechnik, Herrshing, Germany). Twelve male and 12 female dogs, including this female, were euthanized by intravenous injection of sodium pentobarbital (Somnopentyl, Kyoritsu Pharmaceutical Co., Ltd., Tokyo, Japan) at the age of 18 months after a 12-month housing period and subjected to pathological examination. Both the eyeballs and the optic nerves were fixed with mixed fixative consisting of phosphate-buffered 3.0% glutaraldehyde and 2.5% formalin solution, while the brains were fixed in phosphate-buffered 10% formalin solution. The adhesion area between the skull and cerebral cortex was fixed in phosphate-buffered 10% formalin solution, followed by decalcification using K-CX AT (Falma, Tokyo, Japan). Following embedding in paraffin, sections were stained with hematoxylin and eosin (HE). Some sections were also stained with Luxol fast blue (LFB), Masson’s trichrome (MT), and Berlin blue (BB) and subjected to immunostaining for Iba1 and glial fibrillary acidic protein (GFAP). For immunohistochemistry, the primary antibodies used for each section were anti-Iba1 antibody (1:100, polyclonal, Wako, Osaka, Japan) and
anti-GFAP antibody (1:100, polyclonal, Abcam, Cambridge, UK). The secondary antibody used was horseradish peroxidase-labeled polymer (Dako Japan Inc., K4002, EnVision+ System, Tokyo, Japan), and antigen retrieval was performed in 10 mM citrate buffer (pH 6.0) using a microwave for 15 min. Electron microscopic examination was performed on the optic nerve embedded in paraffin. After deparaffinization, optic nerve tissues were fixed in 1% osmium tetroxide and embedded in epoxy resin (Oken Shoji, Tokyo, Japan). Ultrathin sections were stained with uranyl acetate and lead acetate and examined under a JEM-1400 transmission electron microscope (Nippon Denshi, Tokyo, Japan).

This dog had malocclusion and a raised area of the skull from the right frontal region to the crown of the head at the time of receiving the animals, and there were no abnormal clinical signs, including neurological symptoms. The ophthalmologic examination of this animal showed a loss of direct light reflex and hypoplasia of the optic disc in the eye at 6 months of age, while the other animals (12 males and 11 females), purchased at the same time, did not show significant change. Macroscopic examination of the animals euthanized at 18 months of age revealed that the right optic nerve showed a slight decrease in diameter compared to the normal one, and a cystic lesion (approximately 20 × 10 × 5 mm) was detected in the cerebrum just below the raised skull. This cystic lesion was continuous with the right ventricle, contained transparent water, which was probably cerebrospinal fluid, and partially adhered to the dura of the parietal bone. The brain
weight was 59.7 g and was lower than that of the other animals purchased at the same time (78.5 g on average), but there was no difference in the body weight between this case and the other animals. Histologically, in the area surrounding the cystic lesion of the cerebrum, the area of the cortex and medulla of the right hemisphere was reduced, and the thickness of the cerebral parenchyma was thin (Fig. 1A). At the adhesion site between the dura of the parietal bone and the cystic lesion (Fig. 1B), the skull thickened and the number of glial fibers and collagen fibers stained blue with MT increased in the cerebral parenchyma (Figs. 1C, 1D). Furthermore, the number of brown pigments positive for BB and microglial cells positive for Iba1 increased in the cerebral parenchyma near the adhesion site (Figs. 1E, 1F, and 1G). There was no change in the number or distribution of GFAP-positive cells, and no abnormalities were found in the optic tract. The left eyeball and optic nerve were normal. Unlike the left eyeball, the right eyeball revealed retinal thinning which was from focal lesions consisting of atrophy of the nerve fiber layer and inner nuclear layer to entire atrophy involving all layers of the retina (Fig. 2). The right optic nerve showed a reduction in the diameter of the optic disc (Figs. 3A, 3B) and an abnormal arrangement of the fibrous connective tissues (Figs. 3C, 3D). Furthermore, the myelin sheath of the right optic nerve was less stained with LFB than that of the normal optic nerve (Figs. 3E, 3F). Electron microscopic examination revealed that the myelin sheath was unclear in the myelinated nerve fibers of the right optic nerve (Figs. 3G, 3H).
Porencephaly is defined as a smooth-walled cavity filled with cerebrospinal fluid within the cerebral cortex and occurs after necrosis of cerebral white matter \(^1\). In some cases of porencephaly in monkeys and dogs, the lesion has been described as characterized by macroscopic findings of the cerebral hemisphere, open channels between the lateral ventricular and arachnoid space, and histopathological expansion of reactive astrocytes and BB-positive cells \(^6,7\). In this study, porencephaly was detected as a cystic lesion opening channel between the lateral ventricular and arachnoid space with cerebrospinal fluid retention in the cerebral hemispheric parenchyma. These macroscopic findings resemble the morphological features of porencephaly that have been reported to date \(^6,7\). In this case, the skull that adhered to the cerebral parenchyma was raised grossly and histologically showed focal thickening. The cerebral parenchymal lesion of the adhesion site appeared to be an obsolete lesion secondary to a previous injury, because increased glial and collagen fibers, hemosiderin deposition, and an increased number of microglia were observed in this case. Vascular abnormalities of the cerebrum have been reported to be generating factors of congenital porencephaly \(^8\), but there was no abnormal vasculature in this case. In dogs, malocclusion has been reported to occur as a result of genetic defects or secondary to trauma to developing maxillofacial structures \(^9\). Taken together, although there were no abnormal findings in the bone structure suggestive of a reparative change following bone fracture, the porencephaly observed in the present study was
likely caused by trauma to the skull during the postnatal stage.

The present case also had abnormalities in the right eyeball and optic nerve. In the optic nerve, the regions less stained with LFB were characterized by hypoplasia of the myelin sheath of myelinated nerve fibers by electron microscopy. Optic atrophy is characterized by a decrease in the number of optic nerve fibers with axonal degeneration and gliosis \(^{10}\). Meanwhile, optic nerve hypoplasia is a decrease in the diameter of the optic nerve accompanied by a decrease in the number of optic nerve fibers and has been reported to occur as a congenital anomaly, especially in dogs \(^{11,12}\). Optic nerve hypoplasia, characterized by loss of the myelin sheath, has also been reported in beagle dogs \(^{13}\). Since there was no decrease in the number of optic nerve fibers, axonal degeneration, or gliosis, and the myelin sheath was unclear in the present case, the case was diagnosed with optic nerve hypoplasia characterized by hypoplasia of the myelin sheath. Localized retinal atrophy of the nerve fiber layer and the inner nuclear layer to retinal atrophy in all layers was observed in the retina. In cases of humans and dogs showing hypoplasia of the optic nerve, a decrease in the retinal ganglion cell layer and a thinning of the nerve fiber layer have been observed \(^{14-16}\). Therefore, this characteristic retinal thinning was probably attributable to optic nerve hypoplasia. Optic nerve hypoplasia can arise from ocular abnormalities, such as microphthalmos, aniridia, coloboma, nystagmus, and strabismus, or cranial abnormalities, such as agenesis of the septum pellucidum, anencephaly, and midline abnormalities of the brain \(^{17,18}\).
Although there is a case report in which hydrocephalus and unilateral optic nerve hypoplasia were observed in a Pekingese, the relationship between the two lesions is unknown.\textsuperscript{19}

Homonymous hemianopia is defined as bilateral ocular abnormalities associated with disorders of the cerebral hemisphere\textsuperscript{20} however, ocular abnormalities were detected in the unilateral eyeball in the present case. The unilateral occurrence in the present case suggests that porencephaly and ocular abnormalities occurred independently.

Porencephaly has already been reported in dogs\textsuperscript{7,21} but not in experimental beagle dogs. In conclusion, this is an extremely rare case of porencephaly and congenital optic nerve hypoplasia, along with independent retinal thinning.

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Figure Legends

Fig. 1. Microscopic photographs of a cystic lesion of the cerebrum. (A) Low magnification of the brain. A large cystic structure (asterisk) forms in the cerebrum. The area within the black circle indicates an adhesion between the cystic lesion and the dura of the skull. HE stain. (B) Adhesion site between the cerebral parenchyma and the dura of the skull. The skull of the adhesion area shows thickening. HE stain. (C) and (D) High magnification of photograph (B). There is an increase in glial fibers (C, HE stain; bar = 100 µm) and collagen fibers (D, MT stain; bar = 100 µm) in the cerebral parenchyma. (E) Microglia-like cells containing brown pigment were observed in the cerebral parenchyma near the adhesion site (arrowhead). The insert shows high magnification of the brown pigment. HE stain; bar = 50 µm. (F) The brown pigments were positive for BB, suggesting hemosiderin. BB stain; bar = 50 µm. (G) Cells with brown pigment were positive for the Iba1 antibody, suggesting microglia. Iba1 immunostaining; bar = 50 µm.

Fig. 2. Microscopic photographs of retinal thinning in the right eyeball. The central area of the retina shows a normal structure. Toward the peripheral area of the retina, the thickness of the retina is reduced, ranging from focal lesions consisting of atrophy of the nerve fiber layer and the inner nuclear layer to entire atrophy involving all layers of the retina. HE stains; bar = 50 µm.
Fig. 3. Microscopic and electron microscopic photographs of a normal optic nerve (A, C, and E: light microscopy, G: electron microscopy) and a slight decrease in diameter of the right optic nerve (B, D, and F: light microscopy, H: electron microscopy). A and B: The diameter of the right optic disc (B) is smaller than that in the normal optic nerve (A). Each insert shows high magnifications of A or B. HE stain; bar = 500 µm. C and D: An abnormal arrangement of fibrous connective tissue is observed in the right optic nerve (D). Each insert shows high magnifications of C or D. MT stain; bar = 200 µm. E and F: The myelin sheath of the right optic nerve (F) is less stained with LFB compared to that of the normal optic nerve (E). LFB stain, bar = 200 µm. G and H: The myelin sheath in the myelinated nerve fibers is unclear in the right optic nerve (H), bar = 2 µm.
Fig. 1
Normal

Right eyeball

| Central retina | Peripheral retina |

Fig.2.
Fig. 3.