NOTE   *Surgery*

Cushing's Disease Complicated with Thrombosis in a Dog

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ABSTRACT. Thrombosis is a potential complication of hyperadrenocorticism (HAC) in dogs. An 8-year-old male Beagle diagnosed with pituitary-dependent HAC had complicated with thrombosis in the caudal vena cava and abdominal aorta, which was treated by hypophysectomy and antithrombotic therapy. After hypophysectomy, hypercortisolemia disappeared and the general condition was also significantly improved. Ultrasonography after hypophysectomy revealed that the thrombus remained in the abdominal aorta, but the thrombus in the caudal vena cava had disappeared. However 692 days after the hypophysectomy, the dog had an acute onset of dyspnea and died. Postmortem examination revealed the presence of thrombi in the abdominal aorta and the pulmonary artery. Observations from this case show that HAC dogs must be attention to thrombosis.
KEY WORDS: canine, hyperadrenocorticism, thrombosis.

Hyperadrenocorticism (HAC) is a common canine endocrine disease. The characteristic symptoms of HAC are polydipsia, polyuria, abdominal distention, and skin lesions such as alopecia and calcification due to excessive cortisol secretion. HAC dogs may have serious and life-threatening secondary complications and concomitant diseases because long-term hypercortisolemia may cause dysfunction in multiple organs. Hypertension, congestive heart failure, diabetes mellitus, pancreatitis, pyelonephritis, glomerulonephritis, and pulmonary thromboembolism have been reported secondary to HAC [27]. An epidemiological study revealed that morbidity of thrombosis was four-fold higher in patients with Cushing's syndrome than in healthy subjects, and hyperactivation of the blood coagulation system is thought to be involved in the occurrence of thrombosis in humans and dogs with Cushing's syndrome [30]. However, only a few studies have reported HAC dogs with thrombosis [5, 6].

An 8-year-old, male Beagle, weighing 17 kg, was presented to a practitioner with complaints of polydipsia (2 liters/day), polyuria, polyphagia, skin lesions, such as calcification and alopecia, and bilateral hindlimb lameness with pain that had persisted for four months. HAC was diagnosed on the basis of clinical symptoms, blood tests, and endocrinological tests using the administration of a synthetic ACTH compound, tetracosactide acetate (0.25 mg/head, intravascular injection (iv)) (Cortrosyn, Daiichi Pharmaceutical Co., Ltd., Japan). Serum cortisol concentration before ACTH stimulation was 8.4 μg/dl (reference range 0.5–6.0 μg/dl), which increased to 64.4 μg/dl 1 hr after stim-

ulation (reference range 6.0–17.0 μg/dl). The case had been treated for three months with mitotane (Opeprim, Yakult Honsha Co., Ltd., Japan) according to the following oral administration protocol: 50 mg/kg, *semel in die* (SID) for 5 consecutive days; 50 mg/kg, SID for 9 days every 3 days; 25 mg/kg, SID twice a week for 2 weeks; and 25 mg/kg, SID once a week for 5 weeks. However, the bilateral hindlimb lameness with pain persisted. Furthermore, a gastrointestinal disorder appeared, which was likely due to the adverse effect of mitotane. Therefore, the case was referred to the Veterinary Medical Teaching Hospital of Nippon Veterinary and Life Science University for further investigation as to the pathogenesis and treatment.

ACTH stimulation test did not produce the elevated serum cortisol concentration (18.3 μg/dl), but cortisol secretion seemed to be suppressed by the previous prolonged administration of mitotane for three months. There was no abnormality in thoracic radiography, electrocardiography, and neurological tests, including postural reactions and spinal reflex, but general examination revealed edema in the bilateral hind legs, a difference in femoral arterial pressure between the right and left, and bilateral hindlimb lameness with pain. From these findings, thrombosis was suspected. In addition to blood coagulation tests, ultrasonography of the abdominal arteries and veins was performed using a 6.5 MHz microconvex transducer (ULOGIQ 500 PRO Series, General Electric Company, Japan). Blood coagulation tests revealed no remarkable abnormality. However, ultrasonography revealed a parenchymal mass image and a nearby mosaic image representing turbulence in the caudal vena cava (Fig. 1a). In addition, a parenchymal mass image was observed in the abdominal aorta (Fig. 1b). These parenchymal mass images were located caudal to the kidney. An

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Before hypophysectomy   **300 days after hypophysectomy**

Caudal vena cava   Abdominal aorta   Caudal vena cava   Abdominal aorta
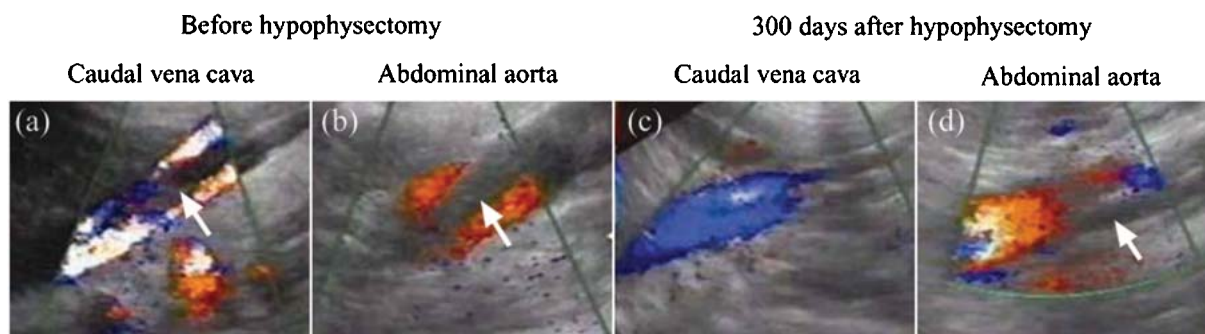

Fig 1.   Images of thrombus by abdominal ultrasonography. Thrombus in the caudal vena cava at the first presentation (a). A parenchymal mass image suspected of thrombus and mosaic echo images adjacent to it were confirmed. Thrombus in the abdominal aorta at the first presentation (b). A parenchymal mass image suspected of thrombus was confirmed. Ultrasonography 300 days after the operation reveals the disappearance of the thrombus in the caudal vena cava observed at the first presentation (c), but the thrombus in abdominal aorta remains (d). Arrows indicate the parenchymal mass.

entanglement and oppression of arteries and veins, often found by the infiltration of pheochromocytoma and adenocarcinoma, were not observed [1, 4]. Ultrasonography revealed that the bilateral adrenals were equally enlarged (right adrenal grand size was 37.3 × 13.4 mm; left adrenal grand size was 37.5 × 11.9 mm) and the liver was mildly enlarged. Pituitary-dependent HAC (PDH) was suspected and magnetic resonance imaging (MRI) of the brain was performed. T1-weighted MRI was conducted with a 1.5 Tesla superconductive magnet (VISART, Toshiba Medical, Japan) using the spin-echo method after an intravenous injection of gadodiamidehydrate (Gd; Omniscan, Daiichi Pharmaceutical Co., Ltd., Japan) at a dose of 0.1 mmol / kg. The scaning protocol of the MRI was as follows: for transverse images, a slice thickness of 2.0 mm, slice gap of 0 mm, time of repetition (TR) of 350 msec, and time of echo (TE) of 15 msec were used; and for sagittal images, a slice thickness of 2.2 mm, a slice gap of 0 mm, TR of 400 msec, and TE of 15 msec were used. As a result, Gd-T1 transverse images revealed the enlarged pituitary gland with the height of 11.7 mm and a Pituitary height/Brain area ratio(PBR; enlarged > 0.31) of 0.69 (Fig. 2a, b) [20]. Based on the results of the endocrinological tests, MRI of the brain, and abdominal ultrasonography, this case was diagnosed as PDH complicated with thrombosis in the caudal vena cava and abdominal aorta.

The animal was treated for 54 days from the first visit with a thrombolytic drug, monteplase (Cleactor, Eisai Co., Ltd., Japan) (400,000 IU, iv, twice), the platelet coagulation inhibitor, heparin sodium (Mitsubishi Pharma Corporation, Japan) (100–200 IU/kg, subcutaneous injection (sc), *bis in die* (BID)) and dipyridamole (Persantin, Nippon Boehringer Ingelheim Co., Ltd., Japan) (1.5–3 mg/kg, per os (po), BID). A cortisol synthesis inhibitor, trilostane (Desopan, Mochida Pharmaceutical Co., Ltd., Japan) (3.5 mg/kg, po, BID) was administered to improve hypercortisolemia for 54 days from the first visit. Although serum cortisol concentration was suppressed after treatment (serum cortisol concentration after ACTH stimulation was 10.4 μg/dl) compared with the

levels observed at the first visit to our hospital, symptoms such as polydipsia, polyuria, alopecia, and hindlimb lameness were not improved. With the pituitary size and the ineffective treatment with mitotane or trilostane, transsphenoidal hypophysectomy was performed on the 55th day after the first visit [24]. For thrombosis, heparin sodium (100 IU/kg, sc, BID for 18 days) and dipyridamole (1.25– 1.5 mg/kg, po, SID- *ter in di*e (TID)) were continued before and after the operation. In addition, after hypophysectomy, hormone replacement therapy was initiated with desmopressin acetate (DDAVP; Desmopressin, Kyowa Hakko Kogyo Co., Ltd., Japan) (0.1–0.2 μg/kg for eye drops, SID-BID), hydrocortisone sodium phosphate (Hydrocortone, Banyu Pharmaceutical Co., Ltd., Japan) (1 mg/kg, iv, TID for 3 days after the operation), prednisolone (Predonine, Shionogi & Co., Ltd., Japan) (0.25–0.5 μg/kg, po, BID) and levothyroxine sodium (Thyradin-S, Aska Pharmaceutical Co., Ltd., Japan) (20–25 μg/kg, po, BID) [15, 16, 23, 24]. The resected pituitary tissue was fixed in 4% paraformaldehyde, embedded in paraffin, and sections (2 μm) were stained with haematoxylin and eosin (HE). As described in the previous report [31], the thin-sectioned tissue were stained immunohistochemically using the peroxidase-labeled antibody method. Anti-ACTH antibody (Monoclonal Mouse Anti-Human Adrenocorticotropin, DAKO Co., California, CA) was used. Foci of enlarged tumor cells with irregular nuclei in the anterior lobe were revealed by HE staining (Fig. 3a). Immunohistochemistry with anti-ACTH antibody showed ACTH-positive cells in the adenoma tissue (Fig. 3b). As a consequence of these findings, we diagnosed the case as a corticotroph pituitary adenoma.

After the surgery, hypercortisolemia disappeared, and water was given *ad libitum* and meals were supplied at 3 days after the surgery. MRI of the brain at 18 days after the surgery confirmed the resection of the pituitary (Fig. 2c, d), but abdominal ultrasonography showed no improvement in the thrombosis. The ACTH stimulation test showed continued low serum cortisol concentrations compared with the results obtained at the first visit to our hospital (serum corti-
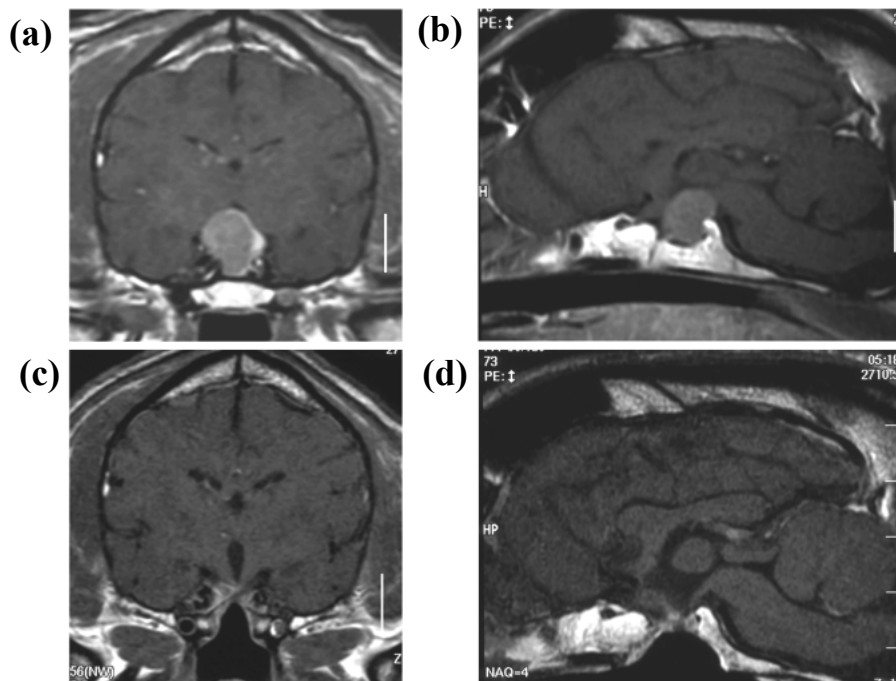
Fig. 2.   MRI of the brain before and after hypophysectomy. A transverse Gd-T1 image before hypophysectomy (a). The height of the pituitary is 11.7 mm and the PBR value (enlarged > 0.31) is 0.69. A sagittal Gd-T1 image before hypophysectomy (b). Resection of the pituitary is confirmed by a transverse (c) and a sagittal (d) Gd-T1 image after hypophysectomy. Bar=10 mm.
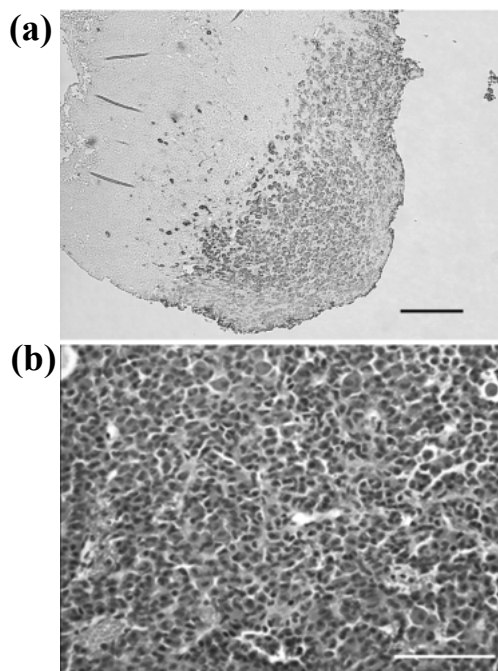

Fig. 3.   Immunohistochemistry of the resected pituitary tissue. (a) Adenoma tissue in the anterior lobe as revealed by HE stain. Bar=100 μm. An enlarged tumor cells with irregular nuclei were observed. (b) Immunostaining of the anterior lobe of the pituitary with anti-ACTH antibody. Bar=200 μm. ACTH-positive cells are observed in the anterior lobe.

sol concentrations after ACTH stimulation: 20 days after the surgery, 4.3 μg/dl; 40 days, 2.4 μg/dl; 210 days, 2.4 μg/dl; 300 days, 4.8 μg/dl, respectively). Ultrasonography at 300 days after the surgery revealed that the thrombus remained in the abdominal aorta, but the thrombus in the caudal vena cava had disappeared (Fig. 1c, d). No specific treatment has been performed for the thrombosis in the abdominal aorta. The general condition of the animal was stable despite persistent polydipsia and polyuria. Lameness and edema in the hind legs were improved. However, 692 days after the surgery, the dog had an acute onset of dyspnoea and died. Postmortem examination revealed the presence of thrombi in the abdominal aorta and the pulmonary artery (Fig. 4). The recurrent pituitary adenoma did not exist.

Canine pituitary tumors are known to expand to their dorsal region with an enlargement of the tumor due to an anatomically incomplete diaphragm of sella [8]. In PDH cases, in addition to symptoms characteristic of HAC, macroadenoma larger than 10 mm in diameter often causes neurologic symptoms by infiltrating and compressing on the hypothalamus and adjacent nerve tissue. Epidemiological studies on the relationship between the size of the pituitary adenoma and neurologic symptoms revealed that all dogs with neurologic symptoms showed a pituitary macroadenoma of 10 mm or larger in diameter [2, 3, 19, 26]. The pituitary adenoma of 11.7 mm in diameter in the present case is categorized as a macroadenoma, but there was no neurologic symptom. The size of the pituitary adenoma and the pres-
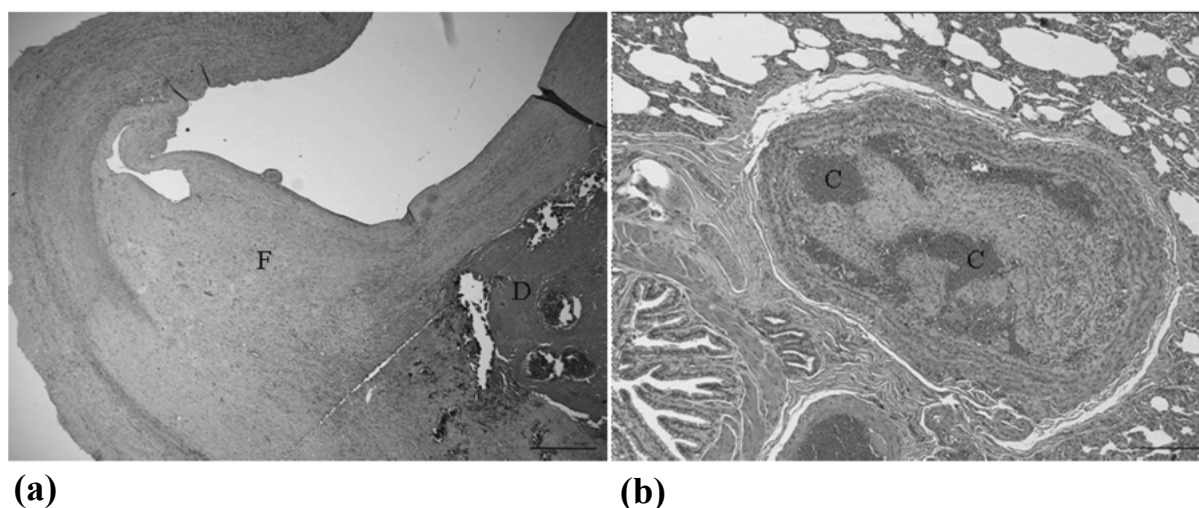
(a)   **(b)**

Fig. 4.   Histopathology of the thrombus formation. (a) An organized thrombus in the aorta. The thrombus is almost replaced by fibrovas-
cular connective tissue (F) except for disorganized area (D). (b) An organized thrombus in a pulmonary artery. The formation of new
canals (C) is seen in the thrombus. HE stain. Bar = 20 μm.

ence or absence of neurologic symptoms greatly influences
the prognosis and survival time in PDH dogs. A study com-
paring survival term between dogs with severe neurologic
symptoms due to macroadenoma and those with mild or no
neurologic symptoms showed that the median survival term
was 50 days and 852 days, respectively [21]. A follow-up
investigation of 150 dogs treated by transsphenoidal hypo-
physectomy showed that the survival rates and recurrence-
free rates for 1–3 years after surgery (The 1-, 2-, and 3-year
survival rate were 84, 76, and 72%, respectively. The 1-,
2-, and 3-year recurrence-free rate were 88, 75, and 66%,
respectively) were superior to those rates after treatment
with mitotane. In addition, the diameter of the pituitary was
shown to affect postoperative survival rates and recurrence-
free rates [13]. In the present case, due to the complication
of thrombosis, risks in surgical procedures of hypophysec-
tomy were a concern. However, since continuous treatment
to reduce circulating cortisol concentrations with mitotane
and trilostane may potentially accelerate the growth of mac-
roadenoma, which may result in neurologic symptoms, sur-
gical treatment was selected in the present case [2, 14, 25,
28, 29].

Since the central diabetes insipidus (CDI) persisted after
the hypophysectomy, two points were considered. (1) Pitu-
itary tumor extension usually occurs in the dorsal direction
and the prolonged mass effect by the tumor on the hypotha-
lamic nuclei may have resulted in damage to Arginine vaso-
pressin (AVP) producing nuclei, such as paraventricular and
supraoptic nuclei, before the hypophysectomy [13]. (2) The
pituitary stalk was sectioned high, which may cause retro-
grade degeneration of paraventricular and supraoptic neu-
rons [22]. It has been reported that the incidence of
permanent CDI is significantly higher in dogs with a large
pituitary tumor than in dogs with a small pituitary tumor
[13]. Therefore, the cause of permanent CDI in this case

was not due to residual or recurrence of pituitary tumor, but
was due to the dimension of pituitary tumor.

It has been reported that coagulation factors II, V, VII,
IX, X, and XII and fibrinogen are increased significantly in
HAC dogs compared with healthy dogs. In addition, anti-
coagulative antithrombins III, as well as a thrombin-anti-
thrombin complex, a marker for subclinical thrombosis, are
significantly decreased in dogs suffering from HAC [11,
17]. Further, *in vitro* studies have shown that corticoster-
oids can reduce fibrinolytic activity by suppressing the
secretion of plasminogen activator by endothelial and liver
or by inducing the release of plasminogen activator inhibitor
type 1 [10]. DDAVP induces an increase in plasma levels of
von Willbrand factor, coagulation factor VIII, and tissue
plasminogen activator [18]. DDAVP administration to pre-
vent hemorrhage intraoperatively does not increase the risk
of thromboembolism, however, continuous administration
to control of diabetes insipidus after surgery may increase
the risk of thrombosis development [12]. Although, we
have experienced some cases that used continuous DDAVP
administration after hypophysectomy, no case showed
thrombosis after the surgery.

We propose that the improvement of hypercortisolemia
by hypophysectomy and continued the antithrombotic ther-
apy after the surgery may lead to the disappearance of the
thrombus formation due to hyperactivation of the blood
coagulation system by hypercoltisolemia in HAC dogs.
However, essential improvement of the thrombosis was not
achieved in this case from the postmortem examination.
While the development of pulmonary thromboembolism is
considered as the cause of death, the actual cause has not
known. However, long-term cortisol excess should be con-
sidered. A link has been reported between cortisol and ath-
erosclerosis, and the development of multiple atherogenic
factors, as a consequence of cortisol excess, is a trigger

mechanism of endothelial damage and artery plaque formation [9]. Moreover, patients with Cushing's disease have a high prevalence of atherosclerosis and maintain increased several cardiovascular risk factors typical of the active phase of the disease even after normalization of circulating cortisol levels for five years [7]. In the present case, the hyperactivation of the blood coagulation system caused by hypercortisolemia may be improved by the hypophysectomy, but the risk of development thrombosis was not reduced.

In conclusion, the risk of thrombosis in HAC dogs may be due not only to hypercoagulation in response to hypercortisolemia, but also to long-term cortisol excess. We did not monitor this possibility sufficiently. Therefore, present or past exposure to cortisol excess should be considered a condition associated with a high risk of thrombosis and such cases should be monitored in a lifelong follow-up.

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