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, tetraacosactide acetate (0.25 mg/head, intravenous 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 stimulation (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
Entanglement and oppression of arteries and veins, often found by the infiltration of pheochromocytoma and adrenocarcinoma, were not observed [1, 4]. Ultrasonography revealed that the bilateral adrenals were equally enlarged (right adrenal grand size was $37.3 \times 13.4 \text{ mm}$; left adrenal grand size was $37.5 \times 11.9 \text{ mm}$) 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 scanning 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 (Persantan, 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 $10.4 \mu g/dl$) 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 die (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 immuno histochemically 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-
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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-
ence or absence of neurologic symptoms greatly influences the prognosis and survival time in PDH dogs. A study comparing 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 hypophysectomy 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 hypophysectomy were a concern. However, since continuous treatment to reduce circulating cortisol concentrations with mitotane and trilostane may potentially accelerate the growth of macroadenoma, which may result in neurologic symptoms, surgical 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) Pituitary tumor extension usually occurs in the dorsal direction and the prolonged mass effect by the tumor on the hypothalamic nuclei may have resulted in damage to Arginine vasopressin (AVP) producing nuclei, such as paraventricular and supraoptic nuclei, before the hypophysectomy [13]. However, since continuous treatment to reduce circulating cortisol concentrations with mitotane and trilostane may potentially accelerate the growth of macroadenoma, which may result in neurologic symptoms, surgical treatment was selected in the present case [2, 14, 25, 28, 29].

Fig. 4. Histopathology of the thrombus formation. (a) An organized thrombus in the aorta. The thrombus is almost replaced by fibrovascular 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.

We propose that the improvement of hypercortisolemia by hypophysectomy and continued the antithrombotic therapy after the surgery may lead to the disappearance of the thrombus formation due to hyperactivation of the blood coagulation system by hypercortisolism 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 considered. A link has been reported between cortisol and atherosclerosis, 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.

REFERENCES