

Studies on the diagnosis and treatment of canine Cushing's disease

Summary of the Doctoral Thesis

Asaka Sato

(Supervised by Professor Yasushi Hara)

Graduate School of Veterinary Medicine and Life Science

Nippon Veterinary and Life Science University

Cushing's disease is a common endocrine disorder in dogs. Approximately 80–85% of Cushing's disease cases in dogs are due to Cushing's disease resulting from an adrenocorticotrophic hormone (ACTH)-secreting pituitary adenoma. However, if the pituitary tumor grows, it becomes to have a detrimental phase as an intracranial space-occupying lesion. Therefore, magnetic resonance imaging (MRI) should be performed upon diagnosis and the treatment should be selected accordingly. For dogs, medication to control excessive cortisol secretion is the current treatment of choice, whereas surgery is the first-line treatment for humans with Cushing's disease. Radiation therapy is also available in veterinary medical science. Among these treatments, transsphenoidal hypophysectomy is the only option for radical cure. However, the indications for transsphenoidal hypophysectomy in dogs have not yet been clarified, and the selection of this technique is mainly at the surgeon's discretion. Therefore, it is important to determine the indications for transsphenoidal hypophysectomy so veterinarians can provide owners with a surgical prognosis. Furthermore, if immunohistological examinations of somatostatin receptor (SSTR) and dopamine D2 receptor (DA2R) in ACTH-secreting pituitary adenomas were available, somatostatin analogs and dopamine agonists, which are reportedly effective against ACTH-secreting pituitary adenomas in humans, might be available when incomplete resection or recurrence would occur in dogs treated with transsphenoidal hypophysectomy.

This study clarified the indications for transsphenoidal hypophysectomy by devising a new classification system reflective of the morphological characteristics of ACTH-secreting pituitary adenomas. Additionally, we determined the SSTR and DA2R expressions in ACTH-secreting pituitary adenomas. Furthermore, bone morphogenetic protein 4 (BMP4) and bone morphogenetic protein receptor (BMPR) expression in ACTH-secreting pituitary adenomas, which is reported to be involved in the mechanism of somatostatin analogs, was also determined.

1. An MRI-based classification system for determining indications for transsphenoidal hypophysectomy in canine pituitary-dependent hypercortisolism

This study aimed to establish a new MRI-based classification system for canine Cushing's disease according to pituitary tumor extent to determine the indications for transsphenoidal hypophysectomy and clarify the prognosis at each disease grade.

We developed a five-point classification system (Grades 1–5) based on tumor extension in the dorsal and craniocaudal directions. Grade 1, no tumor extension beyond the dorsum sellae; Grade 2, tumor extension beyond the dorsum sellae up to the third ventricle but no contact with the optic chiasm or mammillary body; Grade 3, tumor extension beyond the dorsum sellae up to the third ventricle plus contact with the optic chiasm and/or mammillary body but not the interthalamic adhesion; Grade 4, tumor extension beyond the dorsum sellae and contact with the optic chiasm, mammillary body, and interthalamic adhesion; and Grade 5, tumor occupation of the third ventricle. Furthermore, to evaluate blood vessel involvement, tumors of all grades were classified as either Type A, no involvement of the arterial circle of Willis or the cavernous sinus, or Type B, involvement of the arterial circle of Willis or the cavernous sinus.

Complete resection was achieved in three of three Grade 1A cases, three of three Grade 2A cases, 22 of 23 Grade 3A cases, and one of two Grade 3B cases. Resection was incomplete in two of two Grade 4B cases. Grade 5 cases were not indicated for surgery; other therapies were used instead. Recurrence was possible after the first remission or complete resection was achieved and was thus evaluated in 29 rather than 33 cases; recurrence was observed in four of these cases, all of which were classified as Grade 3.

Dogs with Type A, Grade 1–3 Cushing's disease had a good prognosis following transsphenoidal hypophysectomy. However, Type B, Grade 3–5 cases might not be suitable for transsphenoidal surgery.

2. BMP4 and BMPR expression in the pituitary gland of adult dogs in healthy condition and with ACTH-secreting pituitary adenoma

BMP4 reportedly suppresses ACTH secretion, cell differentiation, and tumorigenesis. Furthermore, it is reportedly associated with the function of the somatostatin analogs retinoic acid and ramelteon, which may be effective for treating ACTH-secreting pituitary adenoma. BMP4 is also reportedly expressed in corticotropic cells in the human pituitary gland, although the number of BMP4-positive corticotropic cells was reduced in ACTH-secreting pituitary adenomas.

This study aimed to investigate the expression of BMP4 and its receptors, BMPRI and BMPRII, in the pituitary glands of healthy adult dogs and those with ACTH-secreting pituitary adenomas.

A quantitative polymerase chain reaction analysis showed that the *BMP4* mRNA expression level in the ACTH-secreting pituitary adenoma samples was significantly lower than that in the normal pituitary gland samples ($P = 0.03$). However, there were no statistically significant differences between samples with respect to the mRNA expression levels of the *BMPRIA*, *BMPRIB*, and *BMPRII*. Double immunofluorescence analysis of the normal canine pituitary showed that BMP4 was localized in the thyrotropic ($51.3 \pm 7.3\%$) and not the corticotropic cells. In contrast, BMPRII was widely expressed in the thyrotropic ($19.9 \pm 5.2\%$) and somatotropic cells ($94.7 \pm 3.6\%$), but not in the corticotropic cells. BMP4 and BMPRII were not expressed in the corticotropic cells of ACTH-secreting pituitary adenomas. Moreover, the percentage of BMP4-positive cells was also significantly reduced in the thyrotropic cells of the surrounding normal pituitary tissue obtained from the resected ACTH-secreting pituitary adenoma ($8.3 \pm 7.9\%$) compared to that in normal canine pituitary tissues ($P < 0.001$).

Our study's results revealed a difference in the cellular pattern of BMP4-positive staining in the pituitary gland between humans and dogs and further revealed a BMPRII-positive staining pattern in the normal canine pituitary gland. These species-specific differences in BMP4 should be considered when dogs are used as an animal model for Cushing's disease. Furthermore, somatostatin analogs are reportedly effective for treating Cushing's disease in dogs. However, considering the current results, BMP4 may not be an important factor in terms of somatostatin analog action.

3. Immunohistological analysis of SSTR-2, SSTR-5, and DA2R in the pituitary glands of healthy adult dogs and those with ACTH-secreting pituitary adenomas

In Europe and the USA, the usage of pasireotide, a somatostatin analog, was recently approved for the treatment of adult patients with Cushing's disease for whom pituitary surgery is not a therapeutic option or has not been curative. The dopamine agonists cabergoline and bromocriptine have also been reported as effective for the treatment of Cushing's disease in humans.

This study aimed to clarify the expressions of SSTR2 and SSTR5, which have been reported to suppress hormonal secretion and arrest the cell cycle, as well as DA2R in ACTH-secreting pituitary adenomas.

SSTR2, SSTR5, and DA2R were expressed in the anterior and intermediate lobes of normal canine pituitary glands. However, the positive staining patterns were stronger in the intermediate lobes than the anterior lobes. In the anterior pituitary lobes, SSTR2-, SSTR5-, and DA2R-positive cell ratios in the ACTH-positive cells were $27.0 \pm 8.6\%$, $27.9 \pm 5.9\%$, and $34.0 \pm 9.4\%$, respectively. In contrast, those positive cell ratios in ACTH-positive cells were $97.8 \pm 1.5\%$, $94.1 \pm 4.4\%$, and $96.1 \pm 6.6\%$ in the intermediate pituitary lobes, respectively.

Of the 14 Cushing's disease cases, 11, 12, and six cases expressed SSTR2, SSTR5, and DA2R, respectively. Among these positive-staining cases, four of 11 cases expressing SSTR2 showed strong positive staining in which $>80\%$ of ACTH-positive staining cells co-expressed SSTR2. In addition to SSTR2, seven of 12 cases expressing SSTR5 showed strong positive staining. However, no cases showed DA2R-strong positive staining. Furthermore, four cases showed strong positive staining for both SSTR2 and SSTR5. Two of these cases showed α -melanocyte stimulating hormone-positive staining. This result indicated that these ACTH-secreting pituitary adenomas were derived from the intermediate pituitary lobe.

This study's finding suggested that somatostatin analogs and dopamine agonists may be useful for the treatment of Cushing's disease in dogs according to immunohistological examinations of SSTR2, SSTR5, and DA2R in cases of incomplete resection or recurrence.

This study clarified that dogs with Type A, Grade 1–3 Cushing's disease according to our new classification system were suitable candidates for transsphenoidal hypophysectomy. In these cases, radical cure of Cushing's disease was expected and the dogs would have a good prognosis. Furthermore, these results suggest that the immunohistological staining of SSTR2, SSTR5, and DA2R as well as the use of somatostatin analogs and dopamine agonists would be available for dogs in which incomplete resection or recurrence occurred. However, considering our study findings, BMP4 signaling may not be an important factor with regard to the actions of somatostatin analogs in dogs with Cushing's disease.