Evaluation of progesterone and estrogen receptor expression in 15 meningiomas of dogs and cats

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Objective—To evaluate progesterone and estrogen receptor expression in meningiomas of the CNS in dogs and cats.

Animals—8 dogs (1 of which was treated with gestrinone) and 5 cats with intracranial meningiomas and 2 dogs with spinal cord meningiomas; tissue samples were also obtained from 1 clinically normal dog and 1 clinically normal cat.

Procedure—Meningioma tissue was obtained during surgery or at necropsy; samples were processed for histologic classification and immunohistochemical evaluation of the proportion of tumor cells with progesterone and estrogen receptors. Correlation among receptor expression, tumor grade, and histologic subtypes was determined.

Results—Several histologic subtypes of intracranial meningiomas were detected among tissue samples. In the cats, all intracranial meningiomas were benign. Progesterone receptor immunoreactivity was detected in 14 of 15 meningiomas. Progesterone receptor expression was identified in > 80% of cells in 8 intracranial meningiomas (4 dogs and 4 cats) and 2 spinal cord meningiomas. In samples of malignant transitional and granular cell meningiomas in dogs, progesterone receptors were detected in 32 and 4.8% of cells respectively. In 1 cat, 38% of tumor cells had progesterone receptors. In a dog treated with gestrinone, no progesterone receptors were detected in the intracranial meningioma. Estrogen receptors were only detected in the tumor of 1 dog.

Conclusions and Clinical Relevance—Results indicate a high proportion of progesterone receptors in cells of meningiomas of the CNS in dogs and cats. Antiprogesterone treatment may have a role in the treatment of unresectable or recurrent meningiomas in dogs and cats. (*Am J Vet Res* 2003;64:1310–1318)

Intracranial meningiomas are the most common primary intracranial tumors in dogs, cats, humans, and rats; they are rare in cows, sheep, and horses.[1] Meningiomas appear to be derived from arachnoid cap cells or arachnoid granulation (projections of arachnoid mater through dura mater into the superior sagittal sinus), particularly from arachnoid cells that are associated with the venous sinus of the dura.[1,2] In dogs and cats, meningiomas are usually histologically benign, but their biological behavior may be malignant.[3,4] In dogs and cats, meningiomas are classified as meningothelial, fibroblastic, transitional, psammomatous, angioblastic, papillary, granular cell, myxoid, or anaplastic.[3] Mild nuclear pleomorphism, rare mitoses among cells, absence of tumor infiltration of the neuroparenchyma, and extensive hemorrhage and necrosis are histologic features of benign biological behavior of this tumor. In contrast, malignant meningiomas have high numbers of cells undergoing mitosis, necrosis, loss of normal cellular architecture, and in rare cases, metastasis.[3,5,6]

In dogs, intracranial meningiomas comprise 33 to 49% of all primary brain tumors and are the second most common tumor of the CNS in this species.[4,7,8] Intracranial meningiomas can develop in dogs from 16 months to 14 years of age; however, dogs that are > 7 years of age or dolichocephalic (eg, breeds such as German Shepherd Dog and Collie) are more frequently affected.[4,9,10] In dogs with intracranial meningiomas, the male-to-female ratio is 0.6, which is similar to that among humans these tumors.[2,11] On gross examination, meningiomas in dogs are usually more friable and red, compared with meningiomas in cats.[1] In contrast to histologic findings in meningiomas of humans and cats, many meningiomas in dogs have areas of focal necrosis with pools of neutrophils and some have invasion along the perivascular spaces around the veins and arteries of the CNS.[1] The attachment of meningiomas to the dura or leptomeninges may be broad (sessile), narrow (pedunculated), or total (meningioma en plaque).[1]

In cats, intracranial meningiomas are the most common primary intracranial tumor and comprise 56% of neoplasms of the CNS in this species.[12-16] These tumors develop mainly in geriatric cats (ie, > 10 years old).[12-16] The development of meningiomas in young cats has been associated with mucopolysaccharidosis type 1.[1] There is no breed predisposition for the development of meningiomas; among affected cats, domestic shorthair and domestic longhair cats are overrepresented, although meningiomas are also reported in Siamese, Persian, and Maine Coon cats.[15,16] There is a slight predominance of males among cats with meningiomas.[15,16] Microscopically, these tumors in cats are much more stereotyped than those in dogs; most are meningotheliomatous or psammomatous, and many have cholesterol deposits.[1] Intracranial meningiomas in cats and humans have similarities; these tumors are

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often fibrotic, benign, and usually do not infiltrate brain tissue.[2,13,16] Multiple meningiomas are reported in 14 and 17% of cats in 2 studies and in as many as 20% of humans in several studies.[2,15-17]

Epidemiologic data from humans suggest that the growth of meningiomas may be influenced by the female sex hormones.[18-24] In humans, hormone receptors are expressed by meningiomas; progesterone receptors are detected in most meningiomas, whereas estrogen receptors are expressed to a lesser extent.[21,22] Results of in vitro and in vivo studies[22-24] indicate that these receptors may be functional; this finding may have clinical implications, particularly with regard to use of antiprogesterone treatment.

Receptors for sex hormones in meningiomas of dogs and cats have also been identified in studies[25,26] that used various techniques and experimental conditions. In 1 study,[26] the receptors were identified via a cytosolic receptor-binding assay used on frozen tissue from brain meningiomas of dogs and cats. More recently, **progesterone** and **estrogen receptors** (**PRs** and **ERs**, respectively) were detected immunohistochemically in intracranial meningiomas of dogs.[25] However, to the authors' knowledge, immunohistochemical analyses of PRs and ERs in spinal cord meningiomas of dogs and intracranial meningiomas of cats have not been performed.

The purpose of the study reported here was to evaluate immunohistochemically the proportion of cells with PRs or ERs in spinal cord meningiomas of dogs and intracranial meningiomas of cats and dogs and investigate the association between the proportions of receptors and variables such as sex, tumor grade and histologic subtype, mitotic index, nuclear pleomorphism, necrosis, and inflammatory response. Because human breast carcinoma tissue is used presently as the positive control for immunohistochemical analyses of PRs and ERs, the use of tissue from the uterus and cortex of the kidneys of a clinically normal dog was investigated as an alternative positive control and additional negative control, respectively.

Materials and Methods

Animals—Eight dogs and 5 cats with intracranial meningiomas and 2 dogs with meningiomas in the cervical portion of the spinal cord were included in this study. This study was performed at the School of Veterinary Medicine, University of Wisconsin; certain animals were evaluated and treated at the Villa S. Francesco neurology referral center in Rome. For each of the animals, a detailed history was collected, and physical and neurologic examinations were performed. All intracranial lesions were investigated via either computed tomography or magnetic resonance imaging; myelography and CSF analysis were performed in the 2 dogs with spinal cord meningiomas. For all animals, a presumptive diagnosis of meningioma was made on the basis of the neuroimaging abnormalities. Meningioma samples in 6 dogs and all 5 cats were collected during surgery and in the remaining 4 dogs at necropsy after euthanasia with an IV injection of pentobarbital.[a] Tissues samples were also obtained from a clinically normal dog and a clinically normal cat. These 2 animals were obtained[b] from a commercial supplier and euthanatized as part of a different study. In these animals, the tissue samples were also collected after euthanasia with pentobarbital.

Tissue samples—Meningioma tissue was obtained from the study animals during surgery or at necropsy. Samples of uterus, kidney, cerebral cortex, and brain meninges were collected post mortem from a clinically normal dog, and samples of cerebral cortex and brain meninges were collected from a clinically normal cat. The human breast carcinoma and uterus samples (tissue sections of 6 m thick cut from formalin-fixed paraffin embedded tissue blocks and mounted on polylysine-coated glass slides) were donated from the Comparative Bioscience Laboratory of University of Wisconsin. All samples were processed for the histologic and immunohistochemical evaluation.

Histologic classification and tumor grade—Meningioma tissue samples were fixed in phosphate-buffered 10% formalin solution and routinely processed for histologic evaluation. Slides prepared from meningioma tissue were evaluated for tumor grade (benign or malignant) and subtype according to the Armed Forces Institute of Pathology-World Health Organization classification.[3] To identify granular cell meningioma, additional ultrastructural and immunohistochemical procedures were applied. A sample of the mass was fixed in 2.5% glutaraldehyde and osmium tetroxide and processed for electron microscopy. Tissue sections from paraffin block were stained with periodic acid-Schiff reaction with and without treatment with diastase, Luxol fast blue, and Perl's stains. A section of the tumor was stained with avidin-biotin complex method[c] for immunohistochemical staining of formalin-fixed, paraffin-embedded tissues. The following antibodies were tested: S-100 protein, glial fibrillary acidic protein, vimentin,[d] neuron specific enolase, and lysozyme.[e] Ultra-thin sections were stained with uranyl acetate and lead citrate and examined with a transmission electron microscope.[27,28]

Other variables assessed included degree of differentiation, mitotic rate, nuclear pleomorphism, invasion of the brain, necrosis, and inflammatory response. Degree of differentiation was categorized as high (well-defined groups of neoplastic cells arranged in lobules or whorls) or low (ill-defined whorls of neoplastic cells with loss of architecture and increased cellularity of the tissue). Mitotic rate was calculated as the number of mitoses/10 hpf [400X]. Nuclear pleomorphism was categorized as none (uniform nuclei observed with prominent nuclei), low (occasional cells observed with larger nuclei that had cytoplasmic invaginations), and high (many larger cells with cytoplasmic invaginations into the nuclei). Invasion was classified as absent (tumor compressing the neuroparenchyma with no brain invasion) or present (extensive infiltration of the neuroparenchyma by neoplastic cells). Necrosis was classified as absent (no histologic evidence of necrosis), mild (detection of occasional foci of necrosis, each involving an area of < 0.5 hpf), or severe (frequent detection of foci of necrosis, each involving an area of > 0.5 hpf). Inflammatory response was categorized as absent (no inflammatory cells detected) or present (few clusters of neutrophils detected).

Immunohistochemical evaluation of PRs and ERs—Immunohistochemical analyses of PRs and ERs were performed with monoclonal supersensitive antibodies against PRs and ERs.[29,f] Tissue samples obtained from the 15 meningiomas and the histologically evaluated normal meninges and cerebral cortex of the clinically normal dog and cat were evaluated for PRs and ERs. The prepared paraffin sections of human breast carcinoma tissue and uterus that were known to contain PRs and ERs were used as positive control samples; these were included in each staining procedure involving study tissue samples. Each staining procedure also included negative control samples in which preimmune serum (provided in the immunohistochemistry kit) was used

instead of the primary antibody. Samples of tissue from the uterus and cortex of the kidneys of a clinically normal dog were also included in each staining procedure to investigate their value as positive and negative controls.

Tissue sections (6 m thick) were cut from formalin-fixed, paraffin-embedded tissue blocks and mounted on polylysine-coated glass slides. The sections were deparaffinized in a xylene bath and hydrated in graded ethanol washes. To improve the immunohistochemical staining, slides were incubated in antigen-retrieval solution[f] provided in the kit and heated for 13 minutes in a microwave oven. The microwave oven was initially set at high power for 3 minutes to achieve rapid boiling of the solution and reset at medium power for 10 minutes. As a result, the oven heating cycle went on and off every 20 to 30 seconds, and the solution boiled for approximately 5 to 10 seconds each cycle.

After removing the slide bath from the microwave oven, the slides were allowed to cool for 20 to 30 minutes. Slides were incubated at 4°C with a peroxidase-blocking agent (0.5% hydrogen peroxide in methanol), followed by incubation at in a humidified chamber for 12 hours with the primary PR and ER antibodies[f] (PR 88 and ER 88, respectively), or the negative control serum. The slides were incubated with the secondary biotinylated supersensitive antibody (provided in the immunohistochemical kit) for 30 minutes; this was followed by incubation with peroxidase-conjugated streptoavidine for 30 minutes at 20°C (room temperature). Lastly, slides were incubated with the substrate-chromagen 3,3′ diaminobenzidine until a noticeable brown color change was seen. Sections were counterstained with hematoxylin stain for 3 minutes, dehydrated in graded ethanol washes, and mounted under coverslips with toluene-base[g] permanent aqueous mounting medium.

Assessment of receptor immunoreactivity—All slides were scanned by 1 investigator (PFA) for immunohistochemically stained tumor cell nuclei, and for each assessment, the investigator was unaware of the tumor subtype classification or the disposition of the animal. The number of stained tumor nuclei/500 cells was determined; this value was converted to a percentage.

Statistical analyses—The percentages of PRs and ERs calculated for the tissue samples were analyzed with regard to grade and histologic subtype of the tumor, mitotic index, nuclear pleomorphism, necrosis, inflammatory response, and sex of the animal. Significant correlations between variables were tested with a 2-tailed Fisher exact test. Values of $P < 0.05$ were considered significant.

Results

Animals—Ten dogs and 5 cats were included in the study. Most dogs were dolichocephalic; there were 4 mixed-breed and 5 purebred dogs (including a Shih Tzu, German Shepherd Dog, English Shepherd, Poodle, Golden Retriever, and Bernese Mountain Dog). Meningiomas of the brain were identified in 8 dogs; of these 8 dogs, 5 were sexually intact females, 2 were sexually intact males, and 1 was a castrated male (female-to-male ratio of 1.7:1). The mean age of the dogs with meningiomas of the brain was 10.25 years (range, 6 to 12 years). Two dogs (a 6-year-old castrated male and a 13-year-old spayed female) had meningiomas of the cervical portion of the spinal cord.

Of the 8 dogs with meningiomas of the brain, the tumor was located in the olfactory bulb (n = 3), at the cranial base (3), or at the cerebellopontine-angle (2). One of the meningiomas at the cerebellopontine angle had a large cystic component. Four of these 8 dogs were surgically treated. Complete macroscopic surgical resection was achieved in 2 dogs with meningiomas in the left olfactory bulb; after 2 years, no signs of recurrence of the tumor were detected. Because of the location and extent of the tumor in 2 dogs (with meningiomas at the cranial base or cerebellopontine-angle), only a debulking procedure was performed; these dogs did not regain consciousness after surgery and died as a result of brain stem complications during the following 24 hours. In 3 dogs, euthanasia was performed because the mass was considered unresectable or the owner did not want to pursue any other forms of treatment. One dog with meningioma at the cranial base was treated medically with a synthetic antiprogesterone drug (gestrinone[h]; 1.25 mg, PO, twice a week) and prednisone (administered at an anti-inflammatory dosage); after 6 weeks, the dog was euthanatized at the owner's request because of lack of clinical improvement. Each of the 2 meningiomas of the cervical portion of the spinal cord was completely excised and the dogs had progressive improvement after surgery. However, 1 of them died suddenly after 10 days (unrelated neurologic cause), whereas the other had no clinical signs of recurrence of the tumor after 3 months.

The 5 cats included in the study were 1 Persian, 2 domestic shorthair, and 2 domestic longhair cats. There were 4 spayed females and 1 castrated male (female-to-male ratio of 4:1). Mean age of these cats was 13.7 years (range, 11 to 16 years).

All 5 cats had meningiomas of the brain; the location of the tumor was parieto-occipital (n = 3), parietal (1), or frontotemporal (1). Via computed tomography, intralesional calcifications were detected in association with the parieto-occipital tumors, and calvarium hyperostosis was detected in the other 2 cats. In 1 cat with a tumor in a parieto-occipital location, the meningioma was extremely large and invaded the caudal fossa through erosion of tentorium cerebellum. All 5 cats were treated surgically. In 3 cats, the tumor was completely excised (as determined macroscopically), and the cats recovered from anesthesia without complication. In the cat with the large tumor in the parieto-occipital location, a firm adhesion of the tumor along the dorsal sagittal sinus was identified at surgery, and a small amount of meningioma was not excised. This cat did not recover from anesthesia and died as a result of brain stem complications that may have developed because of surgical manipulation in the caudal fossa. In 1 cat, the tumor was resected completely, but the cat died as a result of complications with respiration shortly after extubation. After 2 and 2.5 years, 2 cats had no clinical signs of recurrence of the tumor; in 1 of these cats, computed tomography performed at 6 and 9 months after surgery did not reveal evidence of meningioma. One cat died from unrelated neurologic causes after 3 months.

Forebrain lesions in dogs were invariably associated with a history of motor seizure activity or behavioral change. This was in contrast to cats in which a change in behavior was the major neurologic manifestation. In all animals, the clinical signs generally correlated well with the site of the lesion. However, 1 cat circled in the

direction opposite to the location of the lesion, and none of the cats had a history of motor activity seizures. Most animals had been treated with steroids before referral.

Histologic classification and tumor grade—Of the intracranial meningiomas in dogs, 7 were benign and 1 was malignant; the latter was a meningioma that was located in the olfactory bulb. The intracranial meningiomas in dogs were classified as meningothelial (n = 4), transitional (3), and granular cell (1). In the 8 dogs, the intracranial meningiomas had no mitoses or a low mitotic rate (range, 0 to 3 mitoses/10 hpf [400 X]; **Table 1**). In the 5 cats, no mitoses were detected in the intracranial meningiomas, which were considered benign; these tumors were classified as transitional (n = 3), psammomatous (1), and fibroblastic (1). The 2 meningiomas in the cervical portion of the spinal cord in dogs were both classified as transitional with low mitotic rates (0 to 1 mitoses/10 hpf).

Meningothelial meningiomas were characterized by lobules of polygonal cells with ill-defined borders, pale eosinophilic cytoplasm, large round to oval nuclei, and a distinct single nucleolus per cell. The cellular lobules were separated by scanty vascular connective tissue. Transitional meningiomas were composed of a mixed population of meningothelial cells arranged in a whorling pattern and separated by elongated fusiform cells arranged in wavy interlacing fascicle. Psammoma bodies were occasionally observed. Elongated spindle cells arranged in long fascicles and numerous psammoma bodies were consistently observed in the fibroblastic and psammomatous subtypes, respectively. Small clusters of neutrophils were occasionally observed in some of the meningothelial and transitional subtypes in dogs and in the fibroblastic and 1 transitional subtypes in cats. No necrotic areas were detected in the fibroblastic and psammomatous subtypes. The malignant intracranial meningioma was characterized by moderate degree of anisokaryosis, low mitotic activity, lack of fibrous tissue, presence of necrotic areas, and extensive invasion of the cerebral cortex.

Receptor immunoreactivity in meningiomas and control tissues—Assessment of sections after immunohistochemical staining for PRs revealed that 14 of the

Table 1—Results of histologic evaluation and immunohistochemical staining for progesterone receptors (PRs) in samples of 13 intracranial meningiomas and 2 meningiomas of the spinal cord from 10 dogs and 5 cats

	Dogs		Cats	
Variable	**No. of tumors and subtype**	**Total**	**No. of tumors and subtype**	**Total**
Tumor grade				
Benign	4M, 4T, 1G	9	3T, 1P, 1F	5
Malignant	1T	1		0
Degree of differentiation				
Well-defined	4M, 3T, 1G	8	2T, 1P	3
Ill-defined	2T	2	1T, 1F	2
Mitotic rate				
Mitoses ≥ 3/10 hpf	4M, 5T, 1G	10	2T	2
Mitoses > 3/10 hpf		0	1T, 1P, 1F	3
Nuclear pleomorphism				
None	2M, 1T, 1G	4	2T	2
Low	2M, 3T	5	1T, 1P, 1F	3
High	1T	1		0
Invasion*				
Absent	1M, 1T, 1G	3	NE	
Present	1T	1	NE	
Necrosis				
Absent	3M, 1T	4	2T, 1P, 1F	4
Mild	1M, 3T, 1G	5	1T	1
Severe	1T	1		0
Inflammatory response				
Absent	2M, 2T, 1G	5	2T, 1P	3
Present	2M, 3T	5	1T, 1F	2
Percentage of cells with progesterone receptors				
≥ 72%	4M, 3T	7	2T, 1P, 1F	4
11–71%	1T	1	1T	1
0–10%	1T, 1G	2		0

M = Meningothelial meningioma. T = Transitional meningioma. G = Granular cell meningioma. P = Psammomatous meningioma. F = Fibroblastic meningioma. NE = Not evaluated.
*Extent of invasion of neuroparenchyma by tumor cells evaluated only in 4 dogs.

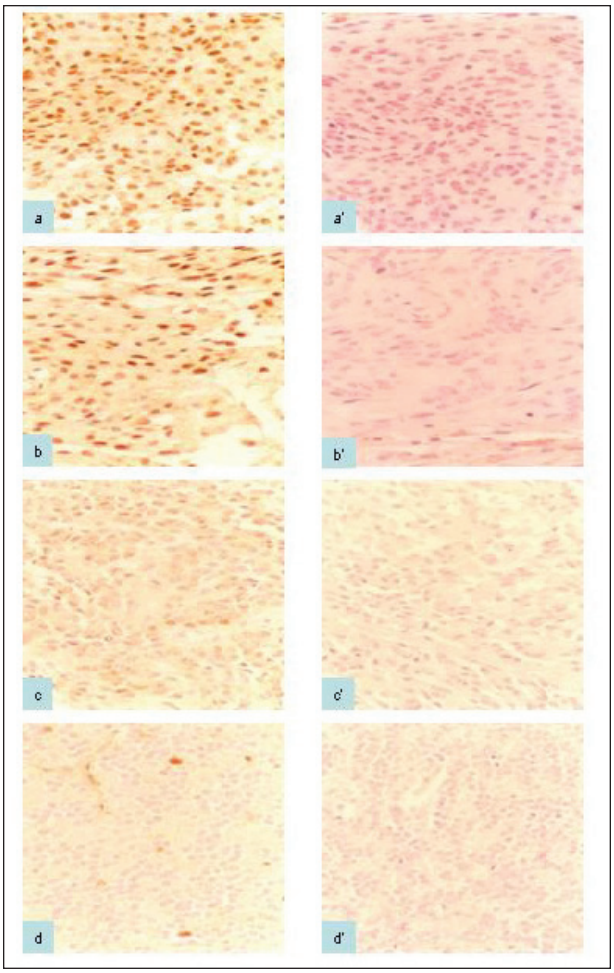

Figure 1—Photomicrographs of sections of intracranial meningiomas of 3 dogs and 1 cat after immunohistologic staining for progesterone receptors (PRs). Notice PR immunoreactivity in > 80% of cell nuclei in sections of a benign meningothelial meningioma of a dog (a) and a benign transitional meningioma of a cat (b). In a malignant meningothelial meningioma of a dog, expression of PRs in cell nuclei was low (32%; c) and in a granular cell meningioma of a dog, only 4.8% of cells were PR-positive (d). a', b', c', and d' = Negative control sections for each sample.

15 meningiomas had cell nuclei that were positively stained. In all of these 14 tissues, PR immunoreactivity was strong, distinct, and localized exclusively to the nuclei of tumor cells; the cytoplasm of tumor cells, connective tissue, or blood vessels in the tissue samples did not react with the stain for PRs. Seven of the intracranial meningiomas in dogs contained cell nuclei that reacted positively after staining for PRs; the tumor from 1 dog had no positively stained nuclei. Tissue from the 2 meningiomas of the cervical portion of the spinal cord also had a positive PR immunoreactivity (**Fig 1**). In 6 of 8 dogs with benign meningothelial or transitional meningiomas, the percentage of positive cells was ≥ 80%. The malignant meningioma had a low number of PR-positive nuclei (32%); in the granular cell meningioma, there were few positive nuclei (4.8%; Fig 1). Among the transitional meningiomas, only the tissue from the dog that had been treated with gestrinone was completely devoid of PRs (**Fig 2**). In the tissue from the 2 meningiomas of the spinal cord, > 80% of the cells were PR-positive. In cats, all 5 intracranial meningiomas had positive PR immunoreactivity (**Table 2**). In 4 cats, the proportion of PR-positive cells in the meningioma tissue was > 80%; in the other cat, this value was 38%. Immunohistochemical staining for ERs in meningiomas yielded negative results for 9 of 10 dogs; meningioma tissue from all cats was negative. One dog with meningothelial meningioma had positive PR and ER immunoreactivity, and the proportion of ER-positive cells was > 80%.

Interestingly, the proportions of PR-positive cells in the meninges of a clinically normal dog and cat were > 80% (Fig 2), but these tissues were devoid of ERs. Immunohistochemical staining for PRs and ERs in samples of cerebral cortex from a clinically normal dog and cat yielded negative results for both immunoreactivity tests, as did the staining of samples of kidney cortex from that dog. Tissue samples of the uterus of the clinically normal dog and human breast carcinoma and uterus also had notable positive PR and ER immunoreactivity (Fig 2). The tissues from the uterus (dog and human) had 100% of cells stained for PRs and ERs; the human breast carcinoma tissue had 100% of cells stained for PRs and > 80% of cells stained for ERs.

Associations between proportion of receptor and tumor grade and histologic subtypes—In 10 dogs, 4 of the intracranial meningiomas were classified as meningothelial; the proportion of PR-positive cells in these tissue samples was > 80% (n = 3) or 72% (1;

Figure 2— Photomicrographs of sections of an intracranial meningioma of a dog and tissue specimens from a clinically normal cat and dog after immunohistologic staining for PRs. Notice the absence of PR immunoreactivity in the section of benign transitional meningioma from a dog that had received antiprogesterone treatment (gestrinone; a). In the meninges of a clinically normal dog (b) and cat (c), PR immunoreactivity was evident in > 80% of cell nuclei. In the uterus of a clinically normal dog, PR immunoreactivity was 100% (d). a', b', c', and d' = Negative control sections for each sample.

Table 2—Characteristics of meningiomas of the CNS in 9 dogs and 5 cats classified by proportion of tumor cells with progesterone receptors

	Proportion of cells with PR immunoreactivity					
	0–10%		11–71%		≥ 72%	
Variable	Dogs	Cats	Dogs	Cats	Dogs	Cats
Tumor grade						
Benign	2	0	0	1	7	4
Malignant	0	0	1	0	0	0
Histologic subtype*						
Meningothelial	0	0	0	0	4	0
Transitional	1	0	1	1	3	2
Granular cell	1	0	0	0	0	0
Degree of differentiation						
Well-defined	2	0	0	0	6	3
Ill-defined	0	0	1	1	1	1
Mitotic rate						
≤ 3/10 hpf	2	0	1	1	7	4
> 3/10 hpf	0	0	0	0	0	0
Nuclear pleomorphism*						
None	1	0	0	0	3	2
Low	1	0	0	1	4	2
High	0	0	1	0	0	0
Necrosis*						
Absent	2	0	0	0	3	4
Mild	2	0	1	1	1	0
Severe	0	0	1	0	0	0
Inflammatory response						
Absent	2	0	0	1	3	2
Present	0	0	1	0	4	2
Sex						
Male	2	0	0	0	2	1
Female	0	0	1	1	5	3

*Significant ($P \leq 0.05$) correlation between variable and percentage of PR-positive cells in dogs.

Table 2). Of the transitional meningiomas, 1 intracranial tumor and the 2 located in the spinal cord had > 80% of PR-positive cells. This proportion was reduced in the other intracranial meningiomas; the malignant transitional meningioma had 32% of PR-positive cells, and the granular cell meningioma had 4.8%. Furthermore, the tissue of the transitional meningioma of the dog that was receiving gestrinone had 0% of PR-positive cells. In cats, all the intracranial meningiomas were benign, and all but 1 had > 80% of PR-positive cells; among these tumors, the exception was 1 transitional meningioma in which the proportion of PR-positive cells was 38% (Table 1 and 2).

Correlation of categorical variables with PR immunoreactivity—A 2-tailed Fisher exact test was used to examine the correlation between proportion of PR-positive cells in tumor tissue and tumor grade and histologic subtype, degree of differentiation, mitotic rate, nuclear pleomorphism, necrosis, inflammatory response, and sex of the animal. Data from the dog treated with gestrinone were not included in the statistical analyses, because the PR status of this tumor may have been modified as a result of the antiprogesterone treatment. In dogs, the proportion of PRs in tumor tissue had a significant correlation with high nuclear pleomorphism, severe necrosis, and with histologic subtype. Degree of differentiation, mitotic index, and inflammatory response did not correlate with the proportion of PR-positive cells; however, all tumors had a mitotic rate < 3. In cats, the proportion of PR-positive cells in tumors was not correlated with any of these variables.

Discussion

The slow growth of meningiomas is associated with insidious and progressive development of neurological dysfunctions.[7,13,16] The clinical signs depend on the location of the tumor, and survival time is short once abnormal neurological signs are observed.[7] Surgical removal is the treatment of choice for operable intracranial meningiomas.[16,30-35] In some instances, complete surgical removal of the tumor can be difficult because of its location and invasiveness. This is particularly true for cranial base meningiomas or those involving important structures such as the cavernous sinus, sagittal sinus, and cranial nerves. After surgical excision, local tumor recurrence is evident in approximately 22% of cats within a follow-up period of 18 to 47 months.[12] The 2-year progression-free survival rate in dogs with meningiomas after surgery and radiation treatment is 68%, and the mean progression-free survival time is 35 ± 7 months.[25] Radiation treatment has been used alone or as adjunctive treatment after cytoreductive surgery or incomplete resection of intracranial meningiomas in dogs.[25,36,37] Gene therapy has been also explored.[38] In humans, treatment for intracranial meningiomas that cannot be excised and that recur following subtotal excision is multimodal.[21,39-44]

The association of sex hormone receptors with meningiomas in humans has been the topic of investigation in many studies. Among humans, the higher frequency of meningiomas in females than in males, the relapse of disease during and at the termination of pregnancy, and the reported epidemiologic link between meningiomas and breast carcinoma have lead to the assumption that gonadal steroid hormones may influence the growth of meningiomas.[29] The abundant expression of PRs in meningiomas in humans is well established.[29,45,46]

In veterinary medicine, few studies have been conducted to assess sex hormone receptors in meningiomas of dogs and cats, and only 1 study has evaluated the prognostic value of PR expression in tumor cells.[25,26] In dogs with incompletely resected meningiomas, 70% of tumor cell nuclei reacted positively for PRs and negatively for ERs after immunohistochemical staining of tissue samples.[25] In the same study, no correlation was found between detection of hormone receptors and age, sex, or sexual status of the dogs, meningioma histologic subtype, or tumor location; however, an inverse association between PRs and cell proliferation was considered indicative of an association between the proportion of PRs and tumor behavior.[25]

In the study reported here, findings indicated a high proportion of PRs and absence of ERs in the nuclei of cells of meningiomas in cats. Similar findings were obtained for meningiomas of the spinal cord in dogs. Results of our study confirm the high number of cells with PRs and significantly lower number of cells with ERs in most of the intracranial meningiomas in dogs. Most intracranial meningiomas in cats and the 2 meningiomas of the spinal cord in dogs had a high number of cells with PRs and a total absence of ERs. All meningiomas evaluated in the study reported here were histologically benign with the exception of a malignant meningothelial intracranial meningioma in 1 dog. In many of the study animals, the neuroanatomical localization of the tumor was considered a major contributing factor in death of the animal. In dogs, most of the benign intracranial meningiomas and the 2 meningiomas of the spinal cord had a high degree of PR expression and were devoid of ERs. Estrogen receptors were detected only in the meningioma of 1 dog (ERs expressed in > 80% of tumor cells), and this tissue also had a high proportion of PR-positive cells (72%). In cats, all intracranial meningiomas were benign; all were devoid of ERs, and all but 1 had high degrees of PR expression (PRs detected in > 80% of tumor cells).

Results of the statistical analyses demonstrated that nuclear pleomorphism, necrosis, and histologic subtype were significantly correlated with the proportion of cells with PRs in meningiomas of dogs. Degree of differentiation, mitotic rate, and inflammatory response did not correlate with the proportion of tumor cells with PRs. In cats, the proportion of tumor cells with PRs was not correlated with any of the variables assessed; it is likely that this finding may have been influenced by the small size of the sample. In the 5 cats, all but 1 of the benign tumors had high proportions of PR-positive cells; in dogs, 7 of the 9 benign meningiomas had high proportions of PR-positive cells (> 72%). The malignant meningioma had a low proportion of PR-positive cells (32%).

These data and the correlation between the proportion of PR-positive tumor cells and nuclear pleomorphism ($P \leq 0.05$) and necrosis ($P \leq 0.05$) confirmed that PR expression in meningiomas of dogs is inversely correlated with aggressive behavior of this type of tumor. A limitation of the study reported here is the inclusion of only 1 malignant tumor, but this corresponds with the low number of malignant meningiomas reported in the literature. In our study, the small number of cats and the lack of malignant meningiomas in those cats precluded evaluation of the association of PR expression in meningiomas of cats and tumor malignancy.

The correlation between the proportion of PR-positive tumor cells and histologic subtype ($P \leq 0.05$) indicated that meningothelial and transitional meningiomas have greater PR expression than that of granular cell meningiomas. The granular cell meningioma evaluated in the study reported here had almost no expression of PRs (4.8%), and this finding is also of particular interest. Granular cell meningiomas in rats have been reported frequently whereas in dogs, there are few reports[27,28,47-49] of this type of tumor involving the intracranial meninges. Findings of recent studies[28,47-49] suggest that intracranial granular cell meningiomas in dogs and rats can be considered a distinct subgroup of benign meningiomas. Granular cell is a descriptive term that is derived from the morphologic appearance of the tumor; however, despite immunocytochemical studies, the histogenesis of this tumor remains controversial.[49] In rats, there is convincing evidence that intracranial granular cell meningiomas originate from meningeal arachnoid cells; some of these tumors may contain only meningeal arachnoid cells, only granular cells, or a mixture of arachnoid and granular cells. It was concluded from this study that granular cell meningioma may be a variant of the meningothelial meningioma.[28] The detection of few PRs in the granular cell meningioma evaluated in our study supports the hypothesis that this type of tumor may have a different histogenesis than that of meningiomas or it can be considered a distinctive pattern of canine meningiomas.

Interestingly, the benign transitional meningioma in the dog treated with gestrinone was completely devoid of PRs. In this dog, the expression of PRs in the tumor before initiation of the antiprogesterone treatment was not known; however, because all other benign transitional meningiomas in dogs that were evaluated in the study of this report had a high proportion of PR-positive cells, we suggest that lack of PRs in this dog could be a result of binding of gestrinone to PRs and subsequent PR downregulation. Alternatively, treatment may have selected for PR-negative cells. Unfortunately, because the tumor in this dog was extremely large, it caused severe compression of the caudal portion of the brainstem and was associated with several cranial nerve abnormalities. The dog was euthanatized, thereby precluding evaluation of the efficacy of long-term treatment with gestrinone (ie, its effectiveness in inducing apoptosis and reducing cell proliferation in the meningioma).[18-22]

Another interesting finding of our study is the high expression of PRs and absence of ERs in meninges of a clinically normal dog and cat, which is in contrast with that of a previous report.[25] The fact that high PR expression was more common in benign meningiomas may indicate that these cells remain similar to normal meningeal cells; decreased or absent PR expression may be a feature of cells with more substantial malignant transformation. However, the high proportion of PR-positive cells in normal meningeal specimens evaluated in the study of this report could also be an incidental finding. Other factors may also be involved in meningioma initiation in dogs and cats, as identified in humans.[50] Unfortunately, evaluation of a large number of normal meningeal specimens was beyond the scope of this study.

Tissue from the uterus of a clinically normal dog had a high degree of PR and ER expression, which is similar to findings in tissues from human uterus and breast carcinoma. Our data suggested that uterine tissue from dogs might be used as a positive control in the immunohistochemical assays used in the study of this report. We also found that the tissue from the cortex of kidneys of dogs is devoid of PRs and ERs; thus, it can be used as additional negative control in those assays.

It has been reported[51] that steroid treatment may affect PR expression, possibly because of the affinity of steroids for binding sites in sex hormone receptors. In contrast, another study[22] indicated no significant difference between PR concentration in meningiomas in humans receiving steroid treatment and in humans who were not receiving steroid treatment. Because most animals in the study of this report were treated with steroids (either before or at the time of the referral) and most of them had a high degree of PR expression, we inferred that steroid treatment did not affect the proportion of PR-positive cells in the meningiomas.

In humans, meningioma growth is enhanced during pregnancy and the luteal phase of the menstrual cycle; at these times, concentration of circulating progesterone is high and suggests that progesterone may be of influence in the growth of meningiomas.[29] In the study of this report, 4 of 5 cats were female, and all cats were castrated or spayed; 6 of 10 dogs were female (1 of which was spayed), and 2 of 4 male dogs were castrated. The study population clearly illustrates that many cats and dogs with meningiomas are neutered. The importance of the association between sex and development of meningiomas is difficult to investigate in dogs and cats because of the confounding effect of neutering. In another study,[25] PR expression in meningioma cells was not associated with sex or reproductive status. In cats, we found no correlation between sex and PR expression; however, because our study included a small number of cats, it would be interesting to repeat the study with a larger group. The serum progesterone concentration was unknown in all the study animals and, to our knowledge, has not been investigated in dogs and cats with meningiomas. The role of serum progesterone concentration in growth of meningiomas in dogs and cats remains to be elucidated.

The regulation of expression of PRs in meningiomas is not understood. The dearth of ERs in 14 of 15 meningiomas evaluated in the study of this report

and the high degree of ER-positive cells in other tumor are unexplained. It is postulated that the autonomous expression of PR in meningiomas is PR promoter-related, but the specific activities of these hormones in the pathogenesis of meningiomas remain unknown.[45] Steroid hormone receptors belong to a large superfamily of nuclear receptors that bind DNA at specific sites to control gene transcription.[50,52] The mechanisms by which steroid receptors modulate the transcription of target genes are under extensive investigation.[54]

Meningiomas in humans express 2 isoforms of the PR (PR-A and PR-B) and an additional PR-78 product.[53] The 2 isoforms seem to have different biological functions; their ratio is highly variable, and progesterone blockade may only be effective in certain subsets of meningiomas.[53] The PR isoforms may also have different affinities for cofactors and other proteins of the transcription machinery.[54] The relative expression of coactivators in meningiomas may contribute to the heterogeneity of hormonal responses observed in vitro and in vivo.[50]

To our knowledge, hormone treatment of meningiomas in veterinary practice has not been investigated. Hormonal treatment has been used in humans with meningiomas with some success. On the basis of that success and on the results of the study of this report and other studies, the efficacy of antiprogesterone treatment in dogs and cats with meningiomas that are unresectable or partially resectable or that recur after partial excision warrants investigation. Surgical removal of benign meningiomas at the cranial base or those that involve critical structures, such as the cavernous sinus, or sagittal sinus is associated with a high mortality rate despite the histologically benign nature of the tumors. For these tumors, surgical techniques and approaches may require reevaluation, and alternative treatments such as administration of antiprogesterone agents could potentially be of benefit.

[a]Euthanasia-6GR Butler, Columbus, Ohio.
[b]Harlan Sprage Dawley, Indianapolis, Ind.
[c]Vector Laboratories, Burlingame, Calif.
[d]Medite Histotechnic Italia, Bergamo, Italy.
[e]Dako, Glostrup, Denmark.
[f]Super Sensitive PR/ER Bio-Genex kit (antibodies PR 88, ER 88, and negative control), Bio-Genex, San Ramon, Calif.
[g]Permount, Fisher Chemical, Fair Lawn, NJ.
[h]Dimetrose, Roussel Laboratories Ltd, Swindon, Wiltshire, UK.

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