Evaluation of matrix metalloproteinase-2 and -9 in the cerebrospinal fluid of dogs with intracranial tumors

Christopher L. Mariani, DVM, PhD; Lindsay B. Boozer, DVM; Alicia M. Braxton; Simon R. Platt, BVM&S; Karen M. Vernau, DVM, MAS; John J. McDonnell, DVM, MS; Julien Guevar, DVM

Objective—To identify matrix metalloproteinase (MMP)-2 and -9 in CSF from dogs with intracranial tumors.

Sample—CSF from 55 dogs with intracranial tumors and 37 control dogs.

Procedures—Latent and active MMP-2 and -9 were identified by use of gelatin zymography. The presence of MMPs in the CSF of dogs with intracranial tumors was compared with control dogs that were clinically normal and with dogs that had idiopathic or cryptogenic epilepsy or peripheral vestibular disease. Relationships between MMP-9 and CSF cell counts and protein were also investigated.

Results—Latent MMP-2 was found in CSF samples from all dogs, although active MMP-2 was not detected in any sample. Latent MMP-9 was detected in a subset of dogs with histologically documented intracranial tumors, including meningiomas (2/10), gliomas (3/10), pituitary tumors (1/2), choroid plexus tumors (5/6), and lymphoma (4/4), but was not detected in any control samples. Dogs with tumors were significantly more likely than those without to have detectable MMP-9 in the CSF, and the presence of MMP-9 was associated with higher CSF nucleated cell counts and protein concentration.

Conclusions and Clinical Relevance—Latent MMP-9 was detected in most dogs with choroid plexus tumors or lymphoma but in a smaller percentage of dogs with meningiomas, gliomas, or pituitary tumors. Detection of MMP in CSF may prove useful as a marker of intracranial neoplasia or possibly to monitor response of tumors to therapeutic intervention.

Objective—To identify matrix metalloproteinase (MMP)-2 and -9 in CSF from dogs with intracranial tumors.

Sample—CSF from 55 dogs with intracranial tumors and 37 control dogs.

Procedures—Latent and active MMP-2 and -9 were identified by use of gelatin zymography. The presence of MMPs in the CSF of dogs with intracranial tumors was compared with control dogs that were clinically normal and with dogs that had idiopathic or cryptogenic epilepsy or peripheral vestibular disease. Relationships between MMP-9 and CSF cell counts and protein were also investigated.

Results—Latent MMP-2 was found in CSF samples from all dogs, although active MMP-2 was not detected in any sample. Latent MMP-9 was detected in a subset of dogs with histologically documented intracranial tumors, including meningiomas (2/10), gliomas (3/10), pituitary tumors (1/2), choroid plexus tumors (5/6), and lymphoma (4/4), but was not detected in any control samples. Dogs with tumors were significantly more likely than those without to have detectable MMP-9 in the CSF, and the presence of MMP-9 was associated with higher CSF nucleated cell counts and protein concentration.

Conclusions and Clinical Relevance—Latent MMP-9 was detected in most dogs with choroid plexus tumors or lymphoma but in a smaller percentage of dogs with meningiomas, gliomas, or pituitary tumors. Detection of MMP in CSF may prove useful as a marker of intracranial neoplasia or possibly to monitor response of tumors to therapeutic intervention.

The prevalence of nervous system tumors in dogs has been estimated to be 14.5/100,000 animals at risk, which is greater than in other domestic species and humans. A variety of primary tumors have been described, including meningiomas, glial cell tumors, choroid plexus tumors, and primary CNS lymphoma. Current options for treatment of brain tumors in canine patients include surgical removal of the tumor, radiation therapy, and chemotherapy. However, chemotherapy remains an unproven modality for brain tumors in veterinary patients. In humans and cats with meningiomas, surgical excision of the tumor is often curative or leads to long-term remission in most cases. Recurrences in these species are often related to a more aggressive cell population, as defined by histopathologic features and a high proliferative index. Most meningiomas in cats and humans are benign, well demarcated, and easily separated from normal brain tissue, facilitating surgical excision. Despite having a similarly benign cellular appearance, many meningiomas in dogs are biologically aggressive, with invasion into adjacent brain parenchyma. Intraoperatively, an obvious demarcation between the tumor and normal tissue is often not discernible. Despite recent advances in operative technique,
recurrence is frequent after surgical treatment alone. Because of this, many veterinary neurologists and oncolgists recommend primary or adjunctive radiation therapy, which appears to improve survival time but is still not curative, and dogs frequently die because of their tumor.11 The reason for this aggressive behavior in canine meningiomas remains unclear. Several groups have examined the proliferative index of these tumors, and although some tumors have high proliferation rates, most have a proliferative index compatible with benign, slow-growing tumors.14,15 Because the aggressive behavior of these neoplasms is associated with invasion of adjacent brain parenchyma,1 potential contributing mechanism is the secretion of enzymes that break down extracellular matrix, allowing subsequent tumor cell invasion. Several enzymes have been associated with this activity in human and canine cancers.16 The MMPs are a family of zinc-dependent endopeptidases that degrade various components of the extracellular matrix. Both membrane-associated and secreted forms of these enzymes have been described, and they are typically released as inactive or latent proenzymes, requiring proteolytic activation after interaction with other proteinases. There are > 20 known MMPs, with a wide variety of substrate specificities.17 The gelatinases MMP-2 (gelatinase A) and MMP-9 (gelatinase B) degrade denatured collagen and type IV collagen as well as other extracellular matrix components, such as laminin, vitronectin, and fibronectin.18 These enzymes are expressed by a number of human and canine tumors.19,20 Expression of MMP-2 and -9 has been reported in malignant gliomas and meningiomas from human patients and has been associated with aggressive behavior.16,21 Previous human studies have correlated expression of MMP-2 and -9 with recurrence22,23 or invasion24,25 of benign meningiomas or with higher meningioma grade.22,24 Matrix metalloproteinase expression has also been detected in other tumors such as primary CNS lymphoma.26 An immunohistochemical study27 of MMP-2 and -9 in canine and feline meningiomas revealed widespread expression of both enzymes, which did not correlate with proliferative activity, tumor grade, or biological aggressiveness. Apart from detecting the presence of these proteolytic enzymes in tumor tissue, identification of these proteins in blood or CSF samples from tumor-bearing patients may provide clinically relevant diagnostic and prognostic information and might suggest new targets for therapeutic intervention. Matrix metalloproteinases have been detected in the blood of human patients with a variety of cancers and have correlated with survival time in many studies.28,29 The presence of activated MMP-2 and -9 in the CSF distinguished patients with intracranial tumors from those without in 1 human study,30 and MMP-9 has been detected in the CSF of patients with malignant gliomas31 and CNS lymphoma.32 Similar to humans, the CSF of clinically normal dogs contains the proenzyme form of MMP-2 but not active MMP-2 or either proenzyme or active MMP-9.33

The purpose of the study reported here was to investigate the presence of MMP-2 and -9 in CSF samples from dogs with intracranial neoplasia. We hypothesized that activated MMP-2 and both latent and activated MMP-9 would be detectable in dogs with a variety of intracranial tumors.

Materials and Methods

Case selection and sample collection—Cerebrospinal fluid was collected with standard techniques from either the cerebellomedullary cistern or the lumbar subarachnoid space. The CSF was collected as part of routine diagnostic testing in animals evaluated for intracranial disease. Routine analysis included nucleated cell and RBC counts, total protein concentration determination, and cytologic examination. In some cases, CSF was obtained immediately after euthanasia without subsequent standard analysis. After collection, the CSF was aliquoted and stored at –80°C until analysis. Informed client consent was obtained for the use of all CSF samples.

Canine patients with a diagnosis of intracranial neoplasia were considered for this study. Patients with a histologic diagnosis obtained from a surgical biopsy specimen or at the time of necropsy were preferentially evaluated for CSF MMPs, although some patients that had diagnoses made with MRI in combination with CSF evaluation were included. Image-diagnosed meningiomas were extra-axial masses with broad dural attachment and intense, homogeneous enhancement after IV contrast administration. Gliomas were variably enhancing masses that were intra-axial in location and had imaging characteristics incompatible with hemorrhage on spin echo and gradient echo sequences. Pituitary tumors were noted to arise from the pituitary gland and were strongly contrast enhancing. Dogs with intracranial lymphoma received that diagnosis via histologic examination or by finding neoplastic lymphoblasts on cytologic examination of the CSF. All other tumors were diagnosed by use of histologic examination after necropsy.

Control animals included clinically normal dogs that were part of unrelated, nonrecovery experimental studies or clinical canine patients with idiopathic (ie, heritable) epilepsy, cryptogenic epilepsy, or peripheral vestibular disease. All dogs with idiopathic or cryptogenic epilepsy or peripheral vestibular disease had unremarkable findings on MRI and CSF evaluation. The results of CSF examination, tumor histologic examination, and glucocorticoid administration were obtained from the medical record.

Gelatin zymography—To detect MMP-2 and -9 in CSF, the method of Bergman et al13 was used, with some modifications. Commercially available, precast 10% polyacrylamide minigels8 with gelatin were used. Five microliters of CSF was combined with an equal volume of zymogram sample buffera and added to each well. A molecular weight ladder8 was added to the first well of each gel. Positive control samples for MMP-2 and -9 were supernatants from cultures of canine osteosarcoma or chondrosarcoma cell lines. Electrophoresis was performed at 100 V for 90 to 120 minutes under denaturing but nonreducing conditions, followed by renaturation in a premixed buffer containing a 2.5% solution of nonionic surfactant8 and by incubation overnight (approx 18 hours) at...
37°C. After incubation, the gels were stained with 0.025% Coomassie brilliant blue stain for 45 minutes, followed by destaining in a methanol (40%) and glacial acetic acid (10%) solution until clear bands were seen (approx 20 to 40 minutes).

Data and statistical analysis—Gels were evaluated for latent and active forms of MMP-2 and -9. The presence or absence of clear bands corresponding to these enzymes was recorded as such, and no attempt was made to quantify the amount of enzyme for each sample. The presence of MMP-9 in dogs with tumors was compared with those without via a Fisher exact test after creation of a 2 X 2 contingency table. D’Agostino and Pearson omnibus testing revealed that most of the CSF cell count and protein data were not normally distributed; therefore, nonparametric tests were used to analyze these data. Cerebrospinal fluid NCC and protein concentration were compared between groups via a Kruskal-Wallis 1-way ANOVA followed by Dunn multiple comparison testing. The CSF NCC, RBC count, and protein concentration were compared between MMP-9–positive and –negative groups with a Mann-Whitney U test. The presence of MMP-9 in dogs receiving or not receiving glucocorticoids was compared via a Fisher exact test. For all comparisons, a value of P < 0.05 was considered significant, and all tests were 2-tailed. A commercially available software program was used for all statistical analyses.

Results

Cerebrospinal fluid samples were obtained from 55 patients with intracranial tumors, which included 21 patients with meningiomas (10 diagnosed via histologic examination and 11 with MRI), 15 with gliomas (10 diagnosed via histologic examination and 5 with MRI), 6 with pituitary tumors (2 diagnosed via histologic examination and 4 with MRI), 6 with choroid plexus tumors (all diagnosed via histologic examination), 4 with lymphoma (3 diagnosed via histologic examination and 1 via cytologic examination), 2 with histiocytic sarcoma (both diagnosed via histologic examination), and 1 with a metastatic hemangiosarcoma (diagnosed via histologic examination). For the 10 patients with gliomas diagnosed histologically, 3 had astrocytomas and 7 had oligodendrogliomas. For controls, samples from 19 clinically normal dogs, 11 dogs with idiopathic or cryptogenic epilepsy, and 7 dogs with peripheral vestibular disease were evaluated (total, 37). All control CSF samples and 50 tumor CSF samples were obtained from the lumbar space. In 2 dogs, the site of CSF collection was not documented.

All CSF samples had latent MMP-2, but active MMP-2 was not found in any samples (Figure 1; Table 1). Matrix metalloproteinase-9 was detected less frequently but was found in CSF samples from all tumor types. Latent MMP-9 was found more frequently than the active form, and all samples with active MMP-9 also had latent MMP-9. Matrix metalloproteinase-9 (in either form) was not found in any of the control samples. A number of samples from dogs with tumors (9/55 [16.4%]) also had bands at much higher molecular weights than expected for either gelatinase (approx 125 and 250 kDa [Figure 2]). These high–molecular weight bands have been documented in previous reports and are considered to represent multimers of the typical gelatinase A and B bands.
latinases or protein aggregates that include MMPs. All samples with high–molecular weight gelatinases also had detectable MMP-9 in the CSF.

Dogs with intracranial tumors were significantly (\(P < 0.001\)) more likely than those without tumors to have detectable MMP-9 in the CSF. Full analysis of CSF (NCC, RBC count, protein concentration, and cytologic evaluation) was available for 54 samples, which comprised the following diagnostic groups: 17 meningiomas (6 confirmed histologically), 9 gliomas (5 confirmed histologically), 3 pituitary tumors (0 confirmed histologically), 3 choroid plexus tumors, 4 lymphoma, 1 histiocytic sarcoma, 10 idiopathic-cryptogenic epileptic cases, and 7 peripheral vestibular disease. Considering all tumors, both the CSF NCC and protein concentration were significantly different between diagnostic groups (\(P = 0.006\) and 0.005, respectively). This analysis did not change significantly when only histologically confirmed tumors were considered (\(P = 0.009\) and 0.007, respectively). Multiple comparison testing revealed that samples from animals with lymphoma had higher NCC than those from animals with meningiomas, idiopathic-cryptogenic epilepsy, and peripheral vestibular disease, and samples from animals with choroid plexus tumors had significantly higher protein concentrations than those with idiopathic-cryptogenic epilepsy. Neoplastic cells were identified on cytologic evaluation of CSF samples from 3 dogs with CNS lymphoma but not in any of the other samples.

For the 54 CSF samples with full analysis, 9 were MMP-9 positive and 45 were MMP-9 negative. All MMP-9–positive samples were from dogs with a histologically confirmed neoplasm. Samples with detectable MMP-9 had significantly higher NCC and protein than MMP-9–negative samples (\(P < 0.001\) for both), but no difference was found in RBC counts (\(P = 0.13\); Table 2). Information on glucocorticoid administration was available for 88 of 92 (95.7%) dogs, and no difference in CSF MMP-9 status was found between dogs receiving or not receiving these medications (\(P = 0.766\)).

Discussion

The present study documented both latent and active forms of MMP-9 in the CSF of a subset of dogs with intracranial tumors. Conversely, none of the clinically normal dogs or dogs with idiopathic epilepsy or peripheral vestibular disease had detectable amounts of this enzyme, and dogs with intracranial tumors were significantly more likely than those without tumors to have detectable MMP-9 in the CSF. Active MMP-2 was not detected in any of the samples. Similar to previous canine reports, latent MMP-2 was present in all CSF samples. High–molecular weight bands (approx 125 and 225 kDa) were also detected in a number of tumor samples, which likely represented MMP-9 in multimeric form or complexed to other proteins such as tissue inhibitors of MMPs or neutrophil gelatinase–associated lipocalin.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of dogs</th>
<th>NCC Mean ± SD</th>
<th>Median</th>
<th>Range</th>
<th>P value</th>
<th>RBC count Mean ± SD</th>
<th>Median</th>
<th>Range</th>
<th>P value</th>
<th>Protein (mg/dL) Mean ± SD</th>
<th>Median</th>
<th>Range</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-9 status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>9</td>
<td>708.2 ± 1,696</td>
<td>12.0</td>
<td>2–5,139</td>
<td>&lt; 0.001</td>
<td>169.3 ± 251.2</td>
<td>38.0</td>
<td>0–775</td>
<td>0.13</td>
<td>88.1 ± 88.5</td>
<td>26.3</td>
<td>20.0</td>
<td>6.1–83.8</td>
</tr>
<tr>
<td>Negative</td>
<td>45</td>
<td>2.2 ± 4.7</td>
<td>1.0</td>
<td>0–28</td>
<td></td>
<td>104.5 ± 292.3</td>
<td>4.5</td>
<td>0–1,850</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Includes 1 dog with lymphoma diagnosed cytologically.

All = Neoplasia histologically confirmed and diagnosed via MRI. HMW = High molecular weight. Histology = Neoplasia only confirmed histologically.

Discussion

The present study documented both latent and active forms of MMP-9 in the CSF of a subset of dogs with intracranial tumors. Conversely, none of the clinically normal dogs or dogs with idiopathic epilepsy or peripheral vestibular disease had detectable amounts of this enzyme, and dogs with intracranial tumors were significantly more likely than those without tumors to have detectable MMP-9 in the CSF. Active MMP-2 was not detected in any of the samples. Similar to previous canine reports, latent MMP-2 was present in all CSF samples. High–molecular weight bands (approx 125 and 225 kDa) were also detected in a number of tumor samples, which likely represented MMP-9 in multimeric form or complexed to other proteins such as tissue inhibitors of MMPs or neutrophil gelatinase–associated lipocalin. Similar bands have been reported in other studies of both canine and human CSF.

Humans also express latent MMP-2 constitutively in the CSF, but the presence of active enzyme or any...
form of MMP-9 is considered abnormal, unless ultra-sensitive techniques are used.\textsuperscript{30,36,39} Several human studies have analyzed MMP concentrations in the CSF of patients with brain tumors. Friedberg et al\textsuperscript{10} found that all patients with malignant gliomas and metastatic CNS disease had latent MMP-9 expression in the CSF. Active MMP-2 and -9 were detected in the CSF of 100% and 67% of patients with leptomeningeal metastasis but only 4% (for both enzymes) of those without metastasis. High–molecular weight gelatinases of 130 and 250 kDa were detected in 92% and 67% of patients, respectively. Another investigation\textsuperscript{31} of patients with malignant gliomas excluded patients with neoplastic cells in the CSF and found similar expression of latent MMP-9 but could not identify active forms of MMP-2 or -9. A study\textsuperscript{35} of CSF in patients with lymphomatous meningitis found latent MMP-9 in all samples with variable concentrations of active MMP-9 and an absence of active MMP-2. Those authors\textsuperscript{36} concluded that CSF MMP-9 correlated with disease activity and was potentially a useful ancillary marker for patients with CSF cytologic results that were suspicious but nondiagnostic for lymphoma. The separation of MMP-9 into latent and active forms may be somewhat artificial as it relates to the potential for enzymatic function in vivo because latent MMP-9 has catalytic activity, even with its pro-peptide intact.\textsuperscript{39,41}

One previous study\textsuperscript{35} has examined the presence of MMP-2 and -9 in the CSF of dogs with intracranial tumors. In that study,\textsuperscript{35} dogs with space-occupying lesions of the CNS diagnosed primarily on the basis of CT or myelography had increased amounts of MMP-2 and -9 (quantified via densitometry) relative to a control group consisting of dogs with idiopathic epilepsy. Latent and active MMP-2 were detected in 100% and 19%, respectively, of CSF samples from patients with mass lesions, although the active MMP-2 bands were not distinctly separated from the latent bands and were considered together for quantification. Latent MMP-9 was detected in 16 of 20 (80%) of samples from dogs with CNS mass lesions, but active MMP-9 was more difficult to detect. Although that study\textsuperscript{35} also used gelatin zymography, there were some methodological differences from the present study. In the present study, a 5-µL volume of noncentrifuged CSF was used, following the original method of Bergman et al,\textsuperscript{33} whereas Turba et al\textsuperscript{10} used 9 µL of supernatant from centrifuged CSF. These differences are unlikely to account for the discrepancy in MMP expression between studies because cellular material in samples in the present study would likely increase MMP expression, which is often robustly produced by leukocytes.\textsuperscript{39} However, we cannot discount the possible effect of inhibitor molecules in the cellular portion of the samples in the present study. Additionally, Turba et al\textsuperscript{10} found that a prolonged incubation (48 hours) increased the sensitivity of detection of both latent and active MMP-9. We attempted to follow this method but did not detect any change in MMP-9 expression in the samples with prolonged incubation (data not shown).

In the present study, a relatively small proportion of the dogs with intracranial meningiomas (2/21 for all tumors; 2/10 histologically diagnosed tumors only), gliomas (3/15 for all tumors; 3/10 histologically diagnosed tumors only), and pituitary tumors (1/6 for all tumors; 1/2 histologically diagnosed tumors only) had detectable MMP-9 in their CSF. This is in contrast to the results of the human study by Friedberg et al,\textsuperscript{10} in which latent MMP-9 was detected in all patients with intracranial neoplasia and activated MMP-2 was identified in a subset of patients with neoplastic cells in the CSF. However, the patients in the Friedberg et al\textsuperscript{10} study had malignant gliomas, metastatic disease, or carcinomatous meningitis, and a substantial proportion (15/39) had identifiable neoplastic cells in the CSF. Obvious neoplastic cells were absent in the CSF from our patient population, with the exception of several of the dogs with lymphoma. The single patient with a putititary tumor and detectable CSF MMP-9 had an unusual, markedly anaplastic neoplasm. Despite this paucity of MMP expression in dogs with meningiomas, gliomas, and pituitary tumors, caution is warranted in extrapolating CSF results to the level of the tumor. It is possible that MMP-2 and -9 may be expressed in these tumors but not in the CSF because of physical separation of these 2 compartments. Indeed, preliminary evidence suggests that this is true for some dogs.\textsuperscript{1}

Compared with dogs with meningiomas and gliomas, a larger proportion of dogs with choroid plexus tumors (5/6), CNS lymphoma (4/4), histiocytic sarcoma (2/2), and metastatic disease (1/1) had detectable MMP-9 in the CSF. Spread of both choroid plexus tumors and lymphoma through the CSF is a well-documented phenomenon,\textsuperscript{42} and leptomeningeal spread of histiocytic sarcoma has also been reported in dogs.\textsuperscript{43,44} In our cases, 3 of 4 lymphoma samples but none of the other samples had neoplastic cells identified on cytologic examination. In many of these samples, pleocytosis as well as high protein concentration were detected, and the source of the MMP-9 was not entirely clear.

Leukocytes in the CSF can express MMP-9 and have the potential to contribute to the activity noted in the zymograms. In a study by Turba et al,\textsuperscript{13} statistical models of the MMP activity determined that the cell count contributed a substantial portion of the MMP-9 activity. Likewise, studies\textsuperscript{35,39,46} of CSF samples from human patients have established a role for leukocytes in the production of MMP-9, although most of these involved patients with inflammatory CNS conditions and not neoplastic disease. We also detected a similar effect, with MMP-9–positive samples having higher nucleated cell counts than did negative samples. However, these results must be interpreted cautiously because only 9 CSF samples with detectable MMP-9 had a complete analysis performed, and 4 of these samples were from dogs with CNS lymphoma. These lymphoma samples had high NCCs and protein concentrations, which likely skewed the analysis. In addition, samples from some dogs with normal cell counts expressed MMP-9. These findings were similar to those of the study by Freidberg et al,\textsuperscript{30} in which samples with pleocytosis were excluded, yet latent MMP-9 was detected in all of the CSF samples. In addition, preliminary evaluation of CSF from dogs with inflammatory CNS disease, most of which had high nucleated cell counts, revealed that a small proportion (3/16) had detectable MMP-9.\textsuperscript{10}
Therefore, there are clearly other sources contributing to the MMP expression, presumably tumor tissue or its associated vasculature. Additional studies evaluating the expression of MMPs in tumor tissue itself with immunohistochemical or other methods may help to clarify this observation. Blood contamination, as indicated by a high RBC count, did not contribute significantly to MMP expression in CSF in this study.

Although a number of investigators have used densitometry analysis of digestion bands in an attempt to quantify MMP concentrations in samples, a number of factors may influence the intensity of bands created by the enzymes.\textsuperscript{47} Matrix metalloproteinase-9 has more gelatinase activity than MMP-2 and may produce a more robust band with similar amounts of protein.\textsuperscript{48} In addition, there is variation in the staining intensity of gels and the clearing created by the gelatins between runs because of factors such as small disparities in experimental conditions (e.g., incubation times and temperature, washing, staining, and destaining conditions) and the amount of copolymerized gelatin in the gels.\textsuperscript{47} As a result, we chose to report the presence or absence of the latent and active enzymes as an all-or-none phenomenon to avoid errors in quantification. Other modalities such as ELISA may be more accurate in quantifying concentrations of MMP, although these methods do not discriminate between the active and latent forms.\textsuperscript{33}

Although not the primary aim of this study, we attempted to investigate the effect of systemic glucocorticoid administration on the expression of MMP-9 in the CSF. Because of a variety of medications, administration schedules, and routes of delivery, we only considered 2 groups, which were dogs that either did or did not receive a systemic glucocorticoid within 1 month prior to CSF collection. No difference in MMP-9 expression was found between these groups. Downregulation of CSF MMP-9 activity with glucocorticoid administration has been detected in several studies\textsuperscript{34-36} of human patients or experimental animals with inflammatory CNS disease. These studies were typically quantitative and failed to reveal complete elimination of the MMP-9 in the CSF, which would be consistent with the results of the present study. Further investigation of the effect of glucocorticoids on canine MMP-9 in CSF is warranted with more rigorous quantification of MMP-9 concentrations and delineation of therapeutic groups.

One limitation of this study was a lack of histopathologic confirmation of neoplasia in all of the patients. Thus, we could not determine with certainty that tumor subgroups were entirely accurate, or indeed, whether some of these dogs had diseases with other etiologies, such as inflammatory disease. However, we followed well-described imaging characteristics of these tumors.\textsuperscript{32} 62% of all dogs with CNS neoplasia did have histologic examination of the tumor performed, and all animals with detectable MMP-9 in the CSF had tumors that were confirmed via histologic examination. In addition, a separate analysis for samples from patients with a histologic diagnosis was performed, which did not substantially alter the results and did not change the conclusions of the study.

Whether MMP expression is related to neoplastic cells in the CSF or facilitates metastatic migration is an intriguing question that warrants future investigation and one that could have both diagnostic and therapeutic implications. The presence of MMP-9 in the CSF of an animal with suspicious but nondiagnostic cytologic changes might prompt additional investigative tests (e.g., immunocytochemical or PCR assay for antigen receptor rearrangements). In addition, assessment of CSF MMP concentration might facilitate monitoring the response of certain tumors to treatment. Although additional studies are required before considering such a strategy, there is evidence from human patients that MMP concentrations in CSF or urine may be correlated with CNS tumor burden and the response to treatment.\textsuperscript{31,33}

Latent MMP-2 was detected in the CSF of all dogs evaluated in the present study, including clinically normal animals, which confirms the findings of previous studies.\textsuperscript{33,35} Active MMP-2 was not detected in any CSF samples and is unlikely to be useful for diagnostic purposes in dogs. Latent and active MMP-9 was detected in a small proportion of dogs with meningiomas, gliomas, and pituitary tumors but in a larger proportion of patients with choroid plexus tumors, intracranial lymphomas, histiocytic sarcomas, and metastatic CNS disease. Samples positive for MMP-9 had higher NCCs and protein concentrations than did negative samples. Although more work is required, assessment of CSF MMP-9 may prove useful in the diagnosis and therapeutic monitoring in a subset of dogs with intracranial neoplasms. Future studies might include investigation of the relationship of MMP-9 status to tumor grade and use as a prognostic indicator, use of a positive MMP-9 test result to trigger additional diagnostic testing for neoplasia in animals with questionable or suspicious CSF cytologic findings, and use of CSF MMP status to assess tumor recurrence in animals receiving definitive treatment. This study might also provide a basis for novel interventions to prevent the progression of certain cancers (e.g., round cell neoplasms and choroid plexus tumors).

\section{Acknowledgments}

We thank Dr. John C. Kinsella, College of Veterinary Medicine, University of Tennessee, for his critical evaluation of the manuscript.

\section{References}


\textsuperscript{b} Ready Gel 15-75 well Zymogram Gels, Bio-Rad Laboratories, Hercules, Calif.

\textsuperscript{c} Zymogram sample buffer, Bio-Rad Laboratories, Hercules, Calif.

\textsuperscript{d} GE Healthcare Rainbow Molecular Weight Markers, Fisher Scientific, Pittsburgh, Pa.

\textsuperscript{e} Gift from Dr. Marlene Hauck, College of Veterinary Medicine, North Carolina State University, Raleigh, NC.

\textsuperscript{f} Zymogram Renaturation Buffer, Bio-Rad Laboratories, Hercules, Calif.

\textsuperscript{g} Triton X-100, Sigma-Aldrich, St Louis, Mo.

\textsuperscript{h} Coomassie blue, Bio-Rad Laboratories, Hercules, Calif.

\textsuperscript{i} Methanol, Fisher Scientific, Pittsburgh, Pa.

\textsuperscript{j} Glacial acetic acid, Fisher Scientific, Pittsburgh, Pa.

\textsuperscript{k} Prism, GraphPad Software Inc, La Jolla, Calif.


References


47. Toth M, Fridman R. Assessment of gelatinases (MMP-2 and MMP-9) by gelatin zymography. In: Brooks SA, Schumacher U,


