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Matrix metalloprotease-9 activity in the cerebrospinal fluid and spinal injury severity in dogs with intervertebral disc herniation

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ABSTRACT

We investigated whether matrix metalloproteinase (MMP)-9 expression in the cerebrospinal fluid (CSF) of dogs with intervertebral disc herniation (IVDH) is associated with the severity of neurological signs and prognosis. CSF from the cisterna magna (C-CSF) and the lumbar spine (L-CSF) of 34 dogs with IVDH was analyzed using zymography. Activity of MMP-9 in L-CSF was detected in 6 of 34 dogs with IVDH, often for more than 7 days after injury. MMP-9 activity was not detected from any of the C-CSF samples. Of the six cases that were MMP-9 positive, all four cases with grade V that had loss of deep pain were non-ambulatory 6 months after treatment. The remaining two cases with grade III and IV could recover mobility. In dogs with grade V thoracolumbar IVDH, MMP-9 expression in the CSF may indicate severe spinal cord injury with poor prognosis.

1. Introduction

In brain and spinal cord injury, matrix metalloproteinases (MMPs), including MMP-9, contribute to early secondary pathogenesis by disrupting the blood–brain/spinal cord barrier and promoting inflammation (Noble-Haeusslein et al., 2002; Rosenberg et al., 1998), oxidative stress (Gasche et al., 2001; Kim et al., 2003), and demyelination (Anthony et al., 1998; Asahi et al., 2001; Chandler et al., 1995; Gijbels et al., 1992). In the spinal cord, expression of MMP-9 has been associated with early secondary injury, being implicated in processes such as blood–spinal cord barrier permeability and neutrophil migration (Duchossoy et al., 2001; Noble-Haeusslein et al., 2002). The mouse model of open spinal cord injury suggests that inhibition of MMP-9 expression improves locomotor outcome (Noble-Haeusslein et al., 2002). MMPs are also involved in injury and disease states in the central nervous system (CNS) (Yong et al., 2001).

Conversely, MMPs can regulate axonal growth during development as well as enhancing axonal regeneration and synaptic plasticity in the CNS (Duchossoy et al., 2001; Yong, 2005; Zhao et al., 2006). Their positive effects on wound healing appear to be based, in part, on their ability to degrade the extracellular matrix (ECM), releasing molecules that regulate angiogenesis (Chang and Werb, 2001).

Intervertebral disc herniation (IVDH) is a frequent problem in dogs and represents the most common cause of acute spinal cord injury with contusion and hemorrhage. Even when severe neurological impairment is present, decompressive surgery provides a good recovery rate, as long as deep pain perception (DPP) is spared. However, the reported prognosis for dogs with loss of DPP varies greatly. In current studies, conventional functional evaluation and duration from onset do not always reflect prognosis (Kazakos et al., 2005; Laitinen and Puerto, 2005; Olby et al., 2003; Ruddle et al., 2006; Scott and McKee, 1999).

Myelography, computed tomography, and magnetic resonance imaging (MRI) are useful in diagnosing spinal cord compression, and some predictive biomarkers of spinal cord degeneration following compressive injury have been also investigated. In structures of the CNS, there is plentiful ECM surrounding the neurons and their dendrites, and this is organized into dense, stable structures known as perineuronal nets (PNNs) (Celio et al., 1998). The components of PNNs such as chondroitin sulfate proteoglycans (CSPGs), hyaluronan, link protein, and tenascin, have attracted recent interest because of their possible involvement in the control of neuronal plasticity and their roles in neuronal regeneration and function (Galtrey and Fawcett, 2007). Moreover, a recent study found the CSF concentration of cartilage oligomeric matrix protein increased in dogs with IVDH (Tokunaga et al., 2010). Therefore, an important step toward defining the pathophysiologic features of secondary spinal injury is to characterize MMP activity in the CSF of dogs with thoracolumbar IVDH.

The purpose of the present study was to determine relationships between the detection of MMP-9 activity in CSF and disease severity in thoracolumbar IVDH.

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2. Materials and methods

2.1. Animal selection and examination

Client-owned dogs were evaluated at a veterinary teaching hospital of Kagoshima University for spinal cord injury resulting from IVDH within 2007–2009. The diagnosis of spinal cord injury was based on appropriate neuroanatomic localization and a history of neurologic dysfunction or substantial neurologic deterioration. Dogs were classified as either ambulatory or non-ambulatory with deep pain sensation present (grade II to IV) or absent (grade V) (Griffiths, 1982; Wheeler and Sharp, 1994) according to the following classification: grade I, spinal hyperesthesia or pain only; grade II, ambulatory paraparesis; grade III, non-ambulatory paraparesis; grade IV, paraplegia with urinary incontinence; grade V, paraplegia, urinary incontinence, and absent deep pain perception.

Dogs included in the study underwent MRI of the thoracolumbar spinal cord using a 0.4-Tesla MRI unit (APERIO Inspire; Hitachi, Japan) to identify the affected disc space. All dogs had clinical evidence of a transverse thoracolumbar (T3-L3) myelopathy, and MRI confirmed a disc extrusion in this region.

The age, breed, and sex of the dogs were recorded. The rate of onset and duration of neurological dysfunction from the onset of signs, as reported by the owner, before presentation to us, were also recorded.

2.2. Treatment of affected dogs

All dogs received general anesthesia according to standard veterinary protocols. CSF was collected from the cerebellomedullary cistern (C-CSF) and L5-6 (L-CSF) space via the placement of a 22 G spinal needle just before surgical decompression. The CSF was centrifuged and stored at −80 °C until analysis. All dogs underwent hemilaminectomy.

All follow-up data on the time to ambulation were based on direct observation or client interviews. A successful outcome was documented if the dog exhibited no urinary incontinence and was able to ambulate unsupported, with only mild residual ataxia and no residual discomfort (neurological grade I to II). An unsuccessful outcome was documented when the dog had a neurological grade of III or higher.

2.3. Gelatin zymography and densitometry

Gelatin zymography was performed using a protocol described previously (Miya et al., 2005). CSF activities of MMP-2 and -9 were analyzed on 7.5% polyacrylamide gels copolymerized with gelatin (1 mg/mL) (Keck et al., 2002). After electrophoresis of a mixture of 0.5 μL of CSF (diluted 3:100 with distilled water) and 5 μL of zymography buffer (4% sodium dodecyl sulfate, 20% glycerol, 1% bromophenol blue, and 2.5 M Tris–HCl, pH 6.8), the sodium dodecyl sulfate was removed from the gels by several washes with 2.5% Triton X-100 in 50 mM Tris–HCl (pH 7.5). Gels were then rinsed with distilled water and the zymograms were subsequently developed for 18 h at 37 °C in refolding buffer (50 mM Tris–HCl, pH 7.5; 0.15 M NaCl; 10 mM $CaCl_2$; and 0.02% NaN_3). Gels were stained with 1% Coomassie brilliant blue for 30 min and then destained in distilled water overnight. A mixture of 5 μL of canine MMP-2 and -9 standard (diluted 3:10 with distilled water) from a lipopolysaccharide-stimulated canine peripheral blood mononuclear cell culture and 5 μL of zymography buffer was loaded into 1 well/plate as the internal standard for serum MMP-2 and -9 activities.

Enzyme activities of samples were quantified as the net intensity of the gelatinolytic activity including the bands of pro- and active-MMP-2 and -9 in each lane, which was determined on the basis of the molecular size of those bands (canine pro-MMP-2, 72 kDa; active-MMP-2, 67 kDa; pro-MMP-9, 92 kDa; active-MMP-9, 83 kDa) using software packages (Science Lab. 2001, Image Gauge Ver. 4.0 and L Process, FujiFilm, Japan). Actual measurements for samples were converted to values relative to the MMP-2 and -9 standards. To determine the intra and interassay variability, fresh aliquots of serum were thawed and 10 measurements were repeated on a single plate (1 gel) for 10 consecutive working days (i.e., a total of 10 gels).

2.4. Statistical analysis

All quantitative data are expressed as mean ± SD. The frequency of detection of MMP-9 activity in CSF of affected dogs was analyzed relative to the neurologic severity and prognosis with Fisher's exact probability test for trend. Values of $P < 0.05$ were considered significant.

3. Results

3.1. Signalment, neurological grade and outcome

Thirty-four dogs with IVDH were included in this study. Details of breed, age, sex, weight, and neurological severity at presentation are shown in Table 1. The majority ($n = 32$; 94.1%) of the dogs were miniature Dachshunds; the remaining breeds were Welsh Corgi ($n = 1$) and Papillon ($n = 1$). Mean ± SD age and body weight of the study population were 6.2 ± 2.44 years and 5.2 ± 1.16 kg, respectively. There were 19 males and 15 females.

All dogs were non-ambulatory at admission; 13 of the 34 lacked deep nociception. Regarding the spinal cord injury scale, 5 dogs

Table 1
Signalment and neurological grade at presentation for 34 dogs with thoracolumbar intervertebral disc herniation (IVDH).

Case	Breed	Age	Sex	BW (kg)	Grade
1.	M. dachshund	13	Male	6.2	4
2.	M. dachshund	5	Male	6.0	4
3.	M. dachshund	5	Male	4.6	4
4.	M. dachshund	5	Female	7.1	4
5.	M. dachshund	5	Female	5.5	4
6.	Papillon	7	Male	6.0	3
7.	W. Corgi	5	Male	14.0	5
8.	M. dachshund	5	Male	7.0	5
9.	M. dachshund	3	Male	6.7	5
10.	M. dachshund	6	Male	7.5	5
11.	M. dachshund	8	Female	7.2	4
12.	M. dachshund	4	Male	5.4	5
13.	M. dachshund	6	Male	4.5	5
14.	M. dachshund	3	Female	2.3	4
15.	M. dachshund	8	Male	5.3	4
16.	M. dachshund	10	Male	5.0	4
17.	M. dachshund	9	Male	8.0	4
18.	M. dachshund	5	Female	3.7	3
19.	M. dachshund	6	Female	5.3	4
20.	M. dachshund	3	Female	6.0	3
21.	M. dachshund	6	Female	5.0	3
22.	M. dachshund	8	Male	4.3	4
23.	M. dachshund	6	Female	4.4	4
24.	M. dachshund	8	Female	4.6	4
25.	M. dachshund	7	Male	5.0	5
26.	M. dachshund	4	Female	4.2	4
27.	M. dachshund	4.5	Male	4.6	5
28.	M. dachshund	5	Female	5.3	5
29.	M. dachshund	6	Female	4.0	5
30.	M. dachshund	6	Female	6.2	5
31.	M. dachshund	4	Female	5.0	3
32.	M. dachshund	13	Male	7.1	5
33.	M. dachshund	8	Male	5.0	4
34.	M. dachshund	4	Male	4.3	5