Acute Phase Proteins in Cerebrospinal Fluid from Dogs with Naturally-Occurring Spinal Cord Injury

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Abstract

Spinal cord injury (SCI) affects thousands of people each year and there are no treatments that dramatically improve clinical outcome. Canine intervertebral disc herniation is a naturally-occurring SCI that has similarities to human injury and can be used as a translational model for evaluating therapeutic interventions. Here, we characterized cerebrospinal fluid (CSF) acute phase proteins (APPs) that have altered expression across a spectrum of neurological disorders, using this canine model system. The concentrations of C-reactive protein (CRP), haptoglobin (Hp), alpha-1-glycoprotein, and serum amyloid A were determined in the CSF of 42 acutely injured dogs, compared with 21 healthy control dogs. Concentrations of APPs also were examined with respect to initial injury severity and motor outcome 42 d post-injury. Hp concentration was significantly higher ($p < 0.0001$) in the CSF of affected dogs, compared with healthy control dogs. Additionally, the concentrations of CRP and Hp were significantly ($p = 0.0001$ and $p = 0.0079$, respectively) and positively associated with CSF total protein concentration. The concentrations of CRP and Hp were significantly higher ($p = 0.0071$ and $p = 0.0197$, respectively) in dogs with severe injury, compared with those with mild-to-moderate SCI, but there was no significant correlation between assessed CSF APP concentrations and 42 d motor outcome. This study demonstrated that CSF APPs were dysregulated in dogs with naturally-occurring SCI and could be used as markers for SCI severity. As Hp was increased following severe SCI and is neuroprotective across a number of model systems, it may represent a viable therapeutic target.

Key words: biomarkers; canine; neurotrauma; secondary injury

Introduction

FORTY PEOPLE PER MILLION are newly diagnosed with spinal cord injury (SCI) in the United States each year and recent estimates suggest 276,000 individuals are living with SCI in the U.S. alone.¹ Following SCI, patients may exhibit impaired ambulation, decreased sensation, bowel and bladder incontinence, and altered sexual function.² Physical disabilities arising from SCI affect individual quality of life, result in substantial fiscal costs to society (\$21.5 billion in the U.S. in 2013), and profoundly increase disability-adjusted life years.³ For example, 57.1% of people report working prior to a SCI, whereas only 11% of individuals are employed one year post-injury.³ Currently, there are no treatments for acute or chronic SCI that have been shown to robustly improve outcomes.²

Canine intervertebral disc herniation (IVDH) is a naturally-occurring acute compressive/contusive SCI that has a high prevalence in pet dogs.⁴ Canine IVDH bears similarities to human SCI

and therefore has been used to validate novel therapies prior to their introduction into clinical trials.^{4–6} As inflammatory mediators of secondary injury are critical therapeutic targets, recent studies have investigated these processes in canine IVDH. Microglial activation, the up-regulation of interleukin-6 (IL-6) and IL-8 messenger RNA, and the expression of IL-8 and monocyte chemoattractant protein-1 (MCP-1) are markers of injury in canine IVDH, as is the case in humans with SCI and rodent models of neurotrauma.^{7–10}

Acute phase proteins (APPs) are produced in a variety of tissues secondary to the release of IL-1, IL-6, and tumor necrosis factor α .¹¹ In humans, APPs measured in the serum and cerebrospinal fluid (CSF) can be used to help facilitate diagnosis, determine prognosis, and measure response to therapy across a multitude of different disease processes.^{12,13} Additionally, APPs function as effector arms in the innate immune response, and may directly induce cytokine release (C-reactive protein, [CRP]), result in damage to endothelium (CRP), or inhibit neutrophil chemotaxis (haptoglobin, [Hp]); as such, they represent targets for novel therapeutic approaches.^{11,14}

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Whereas APPs have been shown to be elevated in the serum and CSF across a number of neurologic diseases,^{15–18} limited data are available relative to APP expression in neurotrauma. In humans with acute traumatic brain injury, a CSF proteomic investigation indicated Hp and other APPs were prominently increased in severely affected individuals.¹⁹ Further, in humans with chronic SCI, a number of investigations have shown increases in serum CRP and potential correlations with ongoing inflammation.^{20,21}

The goal of this study was to examine APPs in the CSF of dogs with and without IVDH-associated SCI, with a particular focus on proteins that have been shown to be critical mediators in other neurological diseases. We anticipated that this model system would be particularly relevant to explore the role of APPs in SCI, as expression of major APPs in humans and dogs are parallel following a variety of inciting events.²² The primary objective of our study was to determine whether dogs with SCI had higher concentrations of APPs in CSF than control dogs. Our second objective was to examine, in dogs with SCI, the correlation between CSF APP concentration and the duration of injury prior to CSF collection, functional status at the time of sampling, and 42 d post-SCI motor outcome.

Methods

Sample size determination

Sample size was determined *a priori* using commercially available software (S-PLUS version 8.2; TIBCO software Inc., Seattle, WA) based on the assumptions of a normal Gaussian distribution of errors, a significance level of 0.05, and a minimum power of 80%. Sample size was estimated to address our primary objective—that in dogs with acute SCI, APP concentration in the CSF is increased, compared with healthy control dogs. Recordable concentrations of CRP have been detected in both healthy (mean, 0.1 $\mu\text{g/mL}$) and neurologically abnormal (mean 0.5–1.59 $\mu\text{g/mL}$) dogs, using methodology parallel to that proposed in this study.²³ Thus, we used the previously reported distribution of detectable CRP as a basis for our power calculation.^{23,24} We elected to use all available control dog ($n=21$) and were able to identify 54 SCI dog samples; however, due to insufficient CSF volume, only 42 SCI samples were included in analysis. This resulted in a power of 88% to detect differences in CSF CRP concentration between groups.

Inclusion criteria

Procedures in dogs with naturally-occurring SCI were performed with owner consent and consisted of only standard medical and surgical care. CSF collected from the cisterna magna, which was obtained during clinical evaluation, was banked with owner consent. Purpose bred dogs were obtained and used with approval from the Texas A&M University Animal Care and Use Committee (AUP 2007-115; AUP 2011-145). All studies adhered to the National Institutes of Health Guide for Care and Use of Laboratory Animals.

A repository of CSF aliquots stored at -80°C and housed at Texas A&M University since December 2009 was searched in July 2014 for samples from dogs with SCI that met the following inclusion criteria: 1) confirmed intervertebral disc herniation (IVDH) between the T3 and L6 vertebrae; 2) neurologic dysfunction of <7 d duration; 3) surgical decompression of the herniated disc with post-operative rehabilitation performed; and 4) complete medical records including neurologic score at admission and follow-up scoring at Day 42 post-surgery. Dogs that were part of ongoing clinical trials or had a myelogram performed as part of pre-operative diagnostics were excluded from this study.

Healthy control CSF samples were collected from purpose-bred dogs with normal physical and neurologic exams, normal complete blood counts, and normal serum biochemical analysis. All CSF

samples from healthy control dogs were collected and stored in the same manner as described for dogs with IVDH-associated SCI and were required to have a normal total nucleated cell count (TNCC; <5 cells/ μL) and total protein concentration (<35 mg/dL).

Sample collection and therapeutic procedures

Dogs with SCI received complete physical examination, neurologic examination, complete blood count, and serum biochemistry prior to anesthesia. Dogs were then pre-medicated with glycopyrrolate (Robinul-V; West-Ward, Eatontown, NJ) and oxymorphone (Numorphan; Endo Pharmaceuticals, Chadds Ford, PA) or hydromorphone (West-Ward). Following pre-medication, dogs were induced with propofol (Rapinivet; Abbott Labs, Chicago, IL) and intubated, and anesthesia was maintained with sevoflurane (SevoFlo; Abbott Labs). Thoracolumbar vertebral column imaging was performed either via magnetic resonance imaging (MRI) or computed tomography. CSF was then collected via needle puncture of the cerebellomedullary cistern, with an aliquot saved and stored at -80°C for further analysis. Following CSF collection and diagnostic imaging, a hemilaminectomy was performed to remove herniated intervertebral disc material and associated epidural hemorrhage. Either gross visualization of the disc material or histopathology was used to confirm the diagnosis of IVDH.

Following surgery, dogs were recovered in the intensive care unit with intravenous fentanyl citrate (Hospira Inc., Lake Forest, IL) analgesia and standard bladder management. Twenty-four hours later, physical rehabilitation consisting of supported overland walking, passive range of motion, and standing strength exercises was initiated. Dogs were released to their owner's care after pain control was achieved via oral analgesics (tramadol hydrochloride; Amneal Pharmaceuticals, Hauppauge, NY) and urine could be voluntarily voided or the bladder manually expressed. Physical rehabilitation exercises were continued by owners for six weeks post-operatively.

Neurologic scoring

Two separate ordinal SCI scoring systems were used in this study and were applied at initial evaluation and 42 d post-surgical recheck.²⁵ Both scoring systems have excellent inter-rater agreement and predict long-term ambulatory outcome in dogs with IVDH-associated SCI. For both assessment tools, dogs were considered ambulatory if they could rise unassisted and take 10 or more steps without falling. Dogs that were non-ambulatory had pelvic limb movements evaluated using tail support. Postural reaction scoring was performed by supporting the dog in a standing position and placing the dorsum of the paw in contact with the ground. Superficial and deep nociception were evaluated by pinching the interdigital webbing and clamping the nail bed with hemostats, respectively. Nociception was considered intact based on demonstration of a behavioral (e.g., orienting to the stimulus, vocalization) or physiological (e.g., tachycardia) response to stimulation.

The modified Frankel score (MFS)²⁵ was used as a coarse ordinal system to stratify injured dogs into groups that parallel those in the American Spinal Cord Injury Association Impairment Scale. The MFS consists of 6 strata where 0=paraplegic with absent deep nociception, 1=paraplegic with deep nociception intact, 2=paraplegic with superficial nociception intact, 3=non-ambulatory paraparetic, 4=ambulatory paraparetic, and 5=spinal hyperesthesia only.

The Texas Spinal Cord Injury score (TSCIS)²⁵ was developed as a more refined system than the MFS and was used for all analyses that did not require stratification into broad functional categories. With this system, individual limbs are assessed independently and given a score based on nociception, gait, and proprioceptive placing. Nociception was scored as 0=absent, 1=deep nociception present but absent superficial nociception, and 2=deep and superficial nociception intact. For gait assessment, scores ranged from 0–6 as follows: 0=no voluntary movement present when

supported; 1=intact limb protraction with no ground clearance; 2=intact limb protraction with inconsistent ground clearance; 3=intact limb protraction with consistent ground clearance; 4=ambulatory with moderate paresis/ataxia (will fall occasionally); 5=ambulatory with mild paresis/ataxia (does not fall even on slick surfaces); and 6=normal gait. Proprioceptive placing was scored as 0 when absent, 1 when delayed (correction to normal posture taking > 1 sec), and 2 when considered normal.

Measurement of acute phase proteins

All CSF samples were thawed and centrifuged at 10,000 g for 1 min prior to APP measurement. Hp measurement was carried out using an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Life Diagnostics, West Chester, PA). Standard curves were established using the following standards: 125.0, 62.5, 31.2, 15.6, 7.8, 3.9, 1.95, and 0.0 ng/mL. Sample optical density was determined using a microplate reader at 450 nm within 15 min of the completion of the assay.

CRP measurement was carried out using an ELISA according to the manufacturer's instructions (Life Diagnostics). Standard curves were established using the following standards: 10.0, 5.0, 2.5, 1.25, 0.625, and 0.0 ng/mL. Sample optical density was determined using a microplate reader at 450 nm within 15 min of the completion of the assay.

Serum amyloid A (SAA) measurement was carried out using an ELISA according to the manufacturer's instructions (Life Diagnostics). Standard curves were established using the following standards: 1000.0, 500.0, 250.0, 125.0, 62.5, 31.25, and 0.0 ng/mL. Sample optical density was determined using a microplate reader at 450 nm within 5 min of the completion of the assay.

Alpha-1-glycoprotein measurement was carried out using an ELISA according to the manufacturer's instructions (Life Diagnostics). Standard curves were established using the following standards: 300.0, 150.0, 75.0, 37.5, 18.8, 9.4, 4.7, and 0.0 ng/mL. Sample optical density was determined using a microplate reader at 450 nm within 15 min of the completion of the assay.

Statistical analysis

All statistical analyses were determined a priori, in consultation with an investigator with expertise in biostatistics (NDC). Baseline population characteristics were summarized using medians and ranges. Acute phase protein concentrations in the CSF of healthy control and SCI dogs were compared using the Wilcoxon rank-sum test. The Wilcoxon rank-sum test was used to determine relationships between CSF acute phase protein concentration in dogs with SCI and SCI severity at admission as measured by the MFS. Spearman's rank correlation coefficient and testing of its statistical significance was used to assess correlation between CSF acute phase protein concentration in dogs with SCI to duration of SCI prior to CSF sampling, cytologic characteristics of CSF, and 42 d post-surgical recovery as measured by TSCIS. Significance was set at <0.05. All analyses were performed using commercially available software (S-PLUS version 8.2; TIBCO software Inc., Seattle, WA).

Results

Population characteristics

Of the 54 SCI dogs identified, 42 had sufficient CSF and clinical data to be included in this study (Table 1). The median age of SCI dogs was 5.75 years (range, 1–12 years) and there were 18 spayed females (43%), four intact females (10%), 12 castrated males (29%), and eight intact males (19%). The three most common breeds represented were dachshund (32; 76%), mixed breed dog (4; 9%), and shih tzu (3; 7%). The median duration of clinical signs prior to CSF collection was 36.45 h (range, 3–181.75 h) and the median MFS prior to acquisition of CSF was 2 (paraplegia; range, 0–5). The TSCIS

TABLE 1. BASELINE POPULATION CHARACTERISTICS IN 42 DOGS WITH SPINAL CORD INJURY (SCI) AND 21 HEALTHY CONTROL DOGS

Variable	SCI dogs (n=42)	Healthy control dogs (n=21)
<i>A. Continuous variables: median (range)</i>		
Age (years)	5.75 (1 to 12)	3 (1 to 4)
Duration of signs prior to admission (h)	36.45 (3.0 to 181.75)	N/A
MFS at admission	2 (0 to 5)	N/A
TSCIS at admission	6 (0 to 20)	N/A
<i>B. Categorical variables: percentage (proportion)</i>		
Sex		
Female intact	10% (4/42)	0% (0/21)
Female spayed	43% (18/42)	14% (3/21)
Male	19% (8/42)	24% (5/21)
Male neutered	29% (12/42)	62% (13/21)
Prevalent breeds		
Dachshund	76% (32/42)	0% (0/21)
Labrador retriever	0% (0/42)	33% (7/21)
Mixed breed	10% (4/42)	24% (5/21)
Level of spinal cord injury		
T12-T13	12% (5/42)	N/A
T13-L1	31% (13/42)	N/A
L1-L2	7% (3/42)	N/A
L2-L3	19% (8/42)	N/A
Other	31% (13/42)	N/A

N/A, not applicable; MFS, modified Frankel score; TSCIS, Texas Spinal Cord Injury Score.

prior to CSF acquisition were as follows: the median nociception score was 4 (range, 0–4), median proprioceptive placing score was 0 (range, 0–3), and median motor score was 2 (range, 0–10). Based on MRI characteristics, the most common location of compressive/contusive injury was overlying the T13-L1 (13; 31%), L2-L3 (8; 19%), and T12-T13 (5; 12%) vertebral articulations. Prior to admission to the hospital, 13 dogs (31%) received glucocorticoids.

There were 21 dogs in the healthy control group (Table 1). The median age was 3 years (range, 1–4 years) and there were three spayed females (14%), 13 castrated males (62%), and five intact males (24%). The three most common breeds represented in the control group were Labrador retriever (7; 33%), beagle hound (5; 24%), and mixed breed dog (5; 24%).

CSF analysis in dogs with SCI

The median TNCC was 2 cells/ μ L (range, 0–107 cells/ μ L), the median RBC was 11 cells/ μ L (range, 0–11,005 cells/ μ L), and the median total protein concentration was 18 mg/dL (range, 9–42 mg/dL; Supplementary Table 1; see online supplementary material at www.liebertpub.com). Of the dogs that had a CSF pleocytosis (defined at >5 cells/ μ L) the median percentage of neutrophils was 49% (range, 0–63%), lymphocytes was 12% (range, 0–63%), monocytes was 25% (range, 0–100%), and eosinophils was 0% (range, 0–3%). None of the dogs in the control group had a CSF pleocytosis.

CSF acute phase proteins are selectively increased following injury

There was a significantly ($p < 0.0001$) greater concentration of Hp in the CSF of dogs with SCI (median, 86.5 ng/mL; range, 0 to

>300), compared with the control group (median, 0.2 ng/mL; range, 0 to >125; Fig. 1D). The concentration of CRP, SAA, and α -1 glycoprotein (A1G) did not significantly differ between injured and healthy control dogs. Within the healthy control dog group, two dogs had CSF CRP concentrations substantially higher than the median reported value and three dogs had CSF Hp concentrations substantially higher than the median reported value. There was no significant difference in any measured APP between injured dogs with post-injury glucocorticoid treatment and those that did not receive glucocorticoids.

CSF APP concentrations in dogs with SCI were correlated with certain commonly measured CSF analytes. CSF CRP concentration was significantly ($p=0.0001$) and positively correlated ($\rho=0.6259$) with CSF total protein concentration (Fig. 2A). Likewise, CSF Hp concentration was significantly ($p=0.0079$) and positively correlated ($\rho=0.4430$) with CSF total protein concentration (Fig. 2B). CSF CRP concentration tended to be higher in dogs with elevated CSF TNCC ($p=0.060$; $\rho=0.29$), but the association was non-significant. There were no other APPs that were significantly correlated with CSF red blood cell count, TNCC, or total protein concentration.

CSF APP concentrations are associated with initial SCI severity but not long-term outcome

Dogs with SCI were stratified based on MFS at the time of CSF sampling into severe (MFS=0; paraplegia with absent pelvic limb nociception) and mild-moderate (MFS>0) SCI. Dogs with severe SCI had a significantly higher concentration of CRP (median, 6.41 ng/mL; range, 0 to >10), compared with dogs with mild-moderate injury (median, 1.1 ng/mL; range, 0 to >10; $p=0.0071$; Fig. 3A). There also was a significantly higher concentration of Hp in dogs with severe SCI (median, >125 ng/mL; range, 0 to >125) versus those with mild-to-moderate SCI

(median, 76.31 ng/mL; range, 0 to >125; $p=0.0197$; Fig. 3D). The concentration of SAA and A1G in the CSF did not differ significantly based on severity of SCI. Additionally, duration of SCI prior to CSF collection was not correlated to CSF APP concentrations.

We examined correlations between CSF APP concentrations and 42 d post-surgical recovery as measured by a validated ordinal pelvic limb motor, postural, and sensory score (TSCIS). There was a modest negative association between CSF CRP concentration and the motor sub-component of the TSCIS 42 d post-injury, although this was non-significant ($\rho=-0.28$; $p=0.0754$; Fig. 4A). There was no significant association between CSF SAA, A1G, or Hp concentration and Day 42 TSCIS motor scores. Additionally, there was no correlation between CSF APP concentrations and TSCIS proprioception or sensory scores.

Discussion

While it is well established that APPs are dysregulated within the central nervous system (CNS) following inflammatory diseases,^{15,16,18} traumatic brain injury,²⁶ and stroke,^{27,28} the authors are unaware of studies in animal models or humans that have assessed APP concentration in the CSF or CNS parenchyma following SCI. Here, we demonstrated that Hp was significantly elevated in the CSF of dogs with naturally-occurring IVDH-associated SCI, compared with healthy control dogs. Additionally, two APPs, CRP, and Hp were significantly and positively correlated with CSF total protein concentration, a marker of blood-spinal cord barrier disruption. Further, dogs with severe SCI had a significantly higher concentration of CRP and Hp in the CSF, compared with dogs with mild-moderate injury. However, there was no correlation between APP concentrations in the CSF and motor, postural, or sensory outcome at Day 42 post-injury.

FIG. 1. Box-and-whiskers plots summarizing C-reactive protein (A), serum amyloid A (B), α -1 glycoprotein (C), and haptoglobin (D) concentrations in the cerebrospinal fluid (CSF) of 42 dogs with spinal cord injury (SCI) and 21 healthy control dogs. Haptoglobin concentration was significantly higher ($p<0.0001$) in the CSF of SCI dogs, compared with healthy control dogs.

FIG. 2. Scatter plots of cerebrospinal fluid (CSF) C-reactive protein (**A**) and haptoglobin (**B**) concentrations versus log₁₀-transformed CSF total protein concentration in 42 dogs with spinal cord injury. CSF C-reactive protein concentration was significantly ($p=0.0001$) and positively correlated ($\rho=0.6259$) with CSF total protein concentration. Likewise, CSF haptoglobin concentration was significantly ($p=0.0079$) and positively correlated ($\rho=0.4430$) with CSF total protein concentration.

Here, we identified Hp as a key APP elevated in the CSF following IVDH-associated SCI in dogs. In a proteomic study performed in the CSF of humans with traumatic brain injury, Hp was likewise prominently expressed.¹⁹ Hp is an α -2-glycoprotein that is produced by the liver and oligodendroglia in response to IL-6 and other inflammatory triggers.^{29,30} In the context of traumatic brain injury, intracranial hemorrhage, and auto-immune encephalitis, Hp has been postulated to exert neuroprotective effects through binding of free hemoglobin.^{31,32} For example, Hp knock-out mice subjected to intracranial hemorrhage have reduced quantities of structural biomarkers, poorer neurologic recovery based on ordinal behavioral scores, and enhanced central nervous system oxidative stress, compared with normal mice receiving an identical injury.³² Hp may exert additional anti-inflammatory effects through suppression of lymphocytes and modulation of macrophage function.³⁰ Thus, there may be an opportunity to favorably manipulate Hp expression through pharmacologic intervention to mitigate secondary SCI and improve outcomes.

CSF Hp and CRP concentrations were positively correlated with CSF total protein concentration. This finding would imply that APPs in the CSF of dogs with IVDH-associated SCI are, in part, increased when the blood–spinal cord barrier is compromised. Both Hp and CRP can be produced locally in the CNS and elution from local sources into the CSF compartment through glymphatic circulation cannot be ruled out.³³ Determining the actual source of CSF APPs is challenging *in vivo*, without sampling peripheral sources of APPs, such as the liver, as well as CNS tissue or conducting experiments that involve protein labeling. For example, previous studies have shown that CSF Hp was increased in neuromyelitis optica independent of Hp concentration in the serum.¹⁵ This could suggest that CNS production of Hp resulted in elevated concentrations of CSF Hp or that passive leakage of Hp across a disrupted blood–brain barrier was the underlying reason for Hp detected in the CSF. Likewise, in humans with traumatic brain injury, elevated concentrations of CSF APPs have been demonstrated without concurrent elevation of these proteins in the serum.¹⁹ As we centrifuged CSF samples prior to APP measurement, it is unlikely that red blood cells or nucleated cells within the CSF directly contributed to APP concentrations.

Additionally, there was a positive correlation between CRP and Hp concentrations in the CSF and SCI severity. Correlations between CSF and serum APP concentration and disease severity have been identified across a broad range of nervous system conditions, including traumatic brain injury,^{26,34} Alzheimer's disease,^{35,36} and auto-immune encephalitis.³⁷ For example, in children with head injury, serum CRP concentrations were higher in patients with severe injury as measured by the Glasgow Coma Scale, than those with mild-moderate injury.²⁶ In the study described here, the association between CSF APPs and SCI severity could be directly related to blood–spinal cord barrier disruption. Across a number of SCI model systems, including dogs with naturally-occurring IVDH, blood–spinal cord barrier disruption is associated with severe injury, as barrier permeability facilitates the transmigration of infiltrating leukocytes and other mediators of secondary injury.^{38–40} Alternatively, correlations between APPs and SCI severity may reflect local production of these proteins or changes in CSF glymphatic circulation following SCI. Finally, concurrent elevation of CSF CRP and Hp concentrations in severe SCI may suggest an environment of concurrent pro- and anti-inflammatory events. Data from rodent contusion models suggest the presence of early pro- and anti-inflammatory signaling in SCI, which may be augmented by polarization of macrophages to M1 and M2 phenotypes, respectively.^{41,42}

We did not find a significant relationship between CSF APP concentration and 42 d post-injury outcome as measured by ordinal motor, sensory, and postural scores, although, a negative correlation between CSF CRP concentration and motor scores approached significance ($\rho=-0.28$; $p=0.0754$). The lack of association between APPs and outcome in this study was not unexpected. First, although CSF Hp concentration was positively correlated with injury severity, Hp has well-recognized neuroprotective properties across an array of disease models. Secondly, while CSF CRP concentration was likewise correlated with SCI severity, it is unclear whether it can directly induce secondary SCI via activation of complement or is merely a bystander. Third, in models of neurotrauma and in humans, APPs act as inconsistent predictive biomarkers.^{19,43–46} Finally, the study reported here was not powered to examine outcome prediction via CSF APP measurement, and thus the number and distribution of cases might not have been sufficient to detect meaningful associations.

FIG. 3. Box-and-whiskers plots summarizing C-reactive protein (A), serum amyloid A (B), α -1 glycoprotein (C), and haptoglobin (D) concentrations in the cerebrospinal fluid (CSF) of dogs with severe (modified Frankel score [MFS]=0, $n=9$) and mild-moderate (MFS>0, $n=33$) spinal cord injury (SCI). Dogs with severe SCI had significantly higher CSF C-reactive protein and haptoglobin concentrations, compared with those with mild-moderate SCI ($p=0.0071$ and $p=0.0197$, respectively).

Here, we used a naturally-occurring, clinically relevant large animal model of compressive/contusive SCI to assess CSF APPs. The study was designed to generate hypotheses by examining concentrations of four CSF APPs and their correlation with injury status, SCI severity, pre-collection timing of SCI, and CSF analytes.

Confirmatory studies investigating promising associations detected here would be necessary to ensure results are generalizable. Such studies would utilize an alternate population of dogs, restrict hypothesis testing, and adjust for potential confounding variables commonly encountered in clinical populations via appropriate

FIG. 4. Scatter plots of C-reactive protein (A), serum amyloid A (B), α -1 glycoprotein (C), and haptoglobin (D) concentrations in the cerebrospinal fluid (CSF) versus Texas Spinal Cord Injury Score (TSCIS; motor component) in 42 dogs 42 d following spinal cord injury. There was no significant association between concentrations of these CSF acute phase proteins and 42 d TSCIS.

statistical approaches (e.g., multivariate logistic regression). Failure to identify associations between APPs and clinical variables in our study could be a function of sample size. We powered our study based on expected CSF CRP concentrations in healthy dogs and dogs with neurological disease; thus, our sample size may not have been sufficient to address other objectives including correlations with clinical variables, such as 42 d motor outcome.

There are certain limitations in using a model system where injuries happen spontaneously and most animals survive SCI, including lack of histologic data, variation in the anatomic level of SCI, some variability in the duration of SCI prior to sampling, and pre-treatment of animals with agents that may modulate immune responses. While CSF APP concentration was not significantly different in dogs with SCI that received pre-collection glucocorticoids, compared with those that did not, this finding does not exclude potential interactions between glucocorticoid administration and other tested variables including CSF analytes and 42 d motor recovery. An additional potential limitation was the use of tail support to assist in visualizing pelvic limb movement as it has been previously established that tail stimulation may facilitate central pattern generator-mediated limb motion.⁴⁷ Finally, we did not have serum samples available from SCI or control dogs included in our study cohort. Concurrent measurement of serum APP concentrations may have permitted additional hypothesis generation particularly with respect to activation of the peripheral innate immune response post-SCI.

Conclusion

Here, we showed that CSF APPs were dysregulated in a naturally-occurring canine model of acute compressive/contusive SCI. Hp was significantly elevated in the CSF of acute SCI dogs, compared with healthy control dogs. Additionally, CRP and Hp significantly and positively correlated to CSF total protein concentration. And finally, the concentration of CRP and Hp were higher in dogs with more severe injury at presentation, although there was no correlation between APP concentrations in the CSF with recovery on Day 42 post-injury scores.

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Author Disclosure Statement

No competing financial interests exist.

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