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Increase in oxidative stress biomarkers in dogs with ascending–descending myelomalacia following spinal cord injury

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ABSTRACT

Multiple biochemical and immunohistochemical tests were performed to elucidate the role of oxidative stress during ascending–descending (A–D) myelomalacia by comparing dogs with this progressive terminal condition to dogs with chronic, focal spinal cord injuries (SCIs) and controls without SCI. Dogs with A–D myelomalacia exhibited increased biochemical markers for oxidative stress, including 8-isoprostane F2α and acrolein, as well as decreased endogenous glutathione with greatest changes occurring at the lesion center. Inflammation, as evident by the concentration of CD18+ phagocytes and hemorrhagic necrosis, was also exacerbated in the lesion of A–D myelomalacic spinal cord compared to focal SCI. The greatest differences in oxidative stress occurred at the lesion center and diminished distally in both spinal cords with A–D myelomalacia and focal SCIs. The spatial progression and time course of A–D myelomalacia are consistent with the development of secondary injury post-SCI. Ascending–descending myelomalacia is proposed as a clinical model that may further the understanding of the role of oxidative stress during secondary injury. Our results indicate that the pathology of A–D myelomalacia is also similar to subacute progressive ascending myelopathy in humans, which is characterized by recurrent neurodegeneration of spinal cord post-injury.

1. Introduction

Ascending–descending (A–D) myelomalacia is a progressive neurodegeneration of the spinal cord that can naturally occur in dogs after severe spinal cord injury (SCI), such as intervertebral disk herniation or other spinal trauma [13,20]. It is unknown why A–D myelomalacia occurs in some dogs with SCI while not in others. Additionally, this always-fatal condition in dogs has not been widely reported in other animal species, except for the possible related human condition of subacute post-traumatic ascending myelopathy [1,26]. In this study, we investigated potential biochemical mediators of A–D myelomalacia in order to better understand secondary injury mechanisms, which occur post-SCI in all mammalian systems.

The pathogenesis of A–D myelomalacia is characterized by hemorrhagic necrosis and liquefaction of the spinal cord tissue, which spreads diffusely over multiple spinal cord segments rostrally and/or caudally from the initial lesion site. As A–D myelomalacia ascends to the nerves that supply the intercostal muscles, and the phrenic nerves in the cervical spinal cord, respiratory paralysis occurs [13]. There is no effective treatment and euthanasia is recommended upon diagnosis to limit suffering of the animal [8]. The incidence of A–D myelomalacia has been documented to range between 14% and 43% of spinal injured dogs — most commonly occurring in chondrodystrophic breeds, which are more susceptible to sustain intervertebral disk herniations than other breeds [11,17,20,27].

Though the gross anatomy and histology of A–D myelomalacia have been well described in the literature [13,17,22,28], a more in depth evaluation of the causal elements that result in some spinal injured dogs to develop A–D myelomalacia while others do not is needed. Histopathological changes in the spinal cord parenchyma and blood vessels during myelomalacia are consistent with the effects of severe ischemia, hemorrhage, inflammation, and other secondary injury mechanisms [12,13,19]. Ascending–descending myelomalacia is typically detected clinically 24 h to several days post-spinal cord trauma, which correlates with peak secondary injury events post-SCI [6,17]. The same causes of traumatic injuries that result in chronic, focal SCIs also initiate A–D myelomalacia. Ascending–descending myelomalacia does not vary according to the severity or type of spinal trauma. Although inflammatory cells, neutrophils and macrophages, are elevated during A–D myelomalacia, infection or other inflammatory events do not seem to play a major role [13]. Thus, secondary injury mechanisms are likely contributing factors of A–D myelomalacia.

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Secondary CNS injury following trauma includes the interrelated factors of, excitotoxicity, calcium entry in to the cell, activation of proteases, vasospasm, hemorrhage, edema, ischemia, inflammation, reperfusion injury, and oxidative stress [6]. An increase in glial fibrillary acidic protein, a marker of astrocyte proliferation and secondary injury has been shown to be elevated in the cerebrospinal fluid of dogs with A–D myelomalacia [24].

In this study, immunohistochemistry and biochemical methods were used to investigate the etiology of A–D myelomalacia with the hypothesis that oxidative stress is a critical mediator of this pathological condition. A major component of secondary injury is oxidative stress [6,7]. The central nervous system (CNS) is particularly vulnerable to oxidative stress due in part to the high energy demand of nervous tissue, a relatively low concentration of endogenous anti-oxidant activity, and large concentrations of polyunsaturated fatty acids in myelin [6]. If oxidative stress becomes exacerbated and unregulated, it could result in progressive necrosis of spinal cord tissue as seen in A–D myelomalacia.

We measured well-known markers of lipid peroxidation and oxidative stress, 8-isoprostane F2α [23] and acrolein [21], and endogenous anti-oxidants, glutathione, [2] as well as comparing phagocytosis between myelomalacic, focally injured, and uninjured spinal cords. We evaluated samples from spinal cord tissue, plasma, urine, and cerebrospinal fluid in these groups of dogs. This study revealed that oxidative stress is an important factor in the development and progression of A–D myelomalacia. From a neuropathological perspective, we are interested in investigating A–D myelomalacia as a unique model of an exacerbated secondary injury response that can lead to further understanding of this complex cascade of events, particularly in regard to the role of oxidative stress during secondary injury after SCI. We believe that due to the 100% mortality rate of dogs that are diagnosed with A–D myelomalacia, such work is extremely clinically relevant in veterinary medicine [20]. We speculate that ascending–descending myelomalacia may also serve as a translational model and lead to novel insights in the understanding of subacute progressive ascending myelopathy, which occurs infrequently in humans post-SCI.

2. Materials and methods

2.1. Animal Use and collection of samples

Dogs admitted to the Purdue University Veterinary Teaching Hospital (PUVTH) with acute spinal cord injury, and whose owners elected humane euthanasia, were eligible for the study with owner informed consent (Table 1). Three groups of dogs were compared in this study including; 1) dogs with SCIs that developed A–D myelomalacia, 2) dogs with focal, static SCIs, and 3) uninjured control animals. Age, breed and weight did not differ between effected and control groups. The first two groups of dogs with SCIs were deep pain negative prior to admittance to the study. Diagnosis of A–D myelomalacia was made by myelogram, MRI, and deteriorating neurological function including progressive loss of cutaneous trunci muscle (CTM) reflex. Care and use of the animals was performed according to standards at the PUVTH. As required for Purdue Animal Care and Use Committee (PACUC) approval, the animals did not undergo any additional procedures as a result of participating in this study. As some samples, particularly urine, were provided by collaborating veterinarians with no additional information other than they were from healthy animals, this is reflected in the unknown age, breed, and sex entries in Table 1.

Urine, cerebrospinal fluid (CSF), and plasma samples were collected during routine testing at the VTH. CSF was collected via cerebellomedullary puncture due to clinical evaluation of the patient or was collected immediately post-mortem prior to autolysis. Urine was collected as part of the clinical work up from an indwelling catheter or by free catch. Spinal cord tissue for histopathologic evaluation and acrolein and endogenous glutathione (GSSH) measurements was excised immediately after euthanasia by dorsal laminectomy of the spine over four or more vertebrae. Depending upon the extent of A–D myelomalacia, lengths of spinal cord tissue were extracted after euthanasia in order to acquire samples at the center of the lesion, at portions showing visible damage due to hemorrhaging, and areas that appeared normal because of the white coloration of myelin.

2.2. Biochemistry

We measured 8-isoprostane F2α as a marker of oxidative stress and lipid oxidation. 8-Isoprostane F2α and other isoprostanes are prostaglandin like compounds formed by the non-enzymatic free radical peroxidation of arachidonic acid [23]. Presence of oxidative stress marker 8-isoprostane F2α, was measured in urine and cerebrospinal fluid CSF samples using a colorimetric enzyme immunoassay performed by Cayman Chemical Company (Ann Arbor, MI). Samples were collected from spinal injured dogs when admitted to PUVTH and immediately frozen and stored at −80 °C prior to evaluation. Frozen samples were then shipped to Cayman Chemical overnight with dry ice. CSF samples were assayed for free F2α isoprostane, and urine samples were sent to Cayman Chemical Company (Ann Arbor, MI) to be assayed for free 8-isoprostane F2α using their

Table 1
A summary of the characteristics of the animals used in this study.

Dog	Condition (Group)	Breed	Age (years)	Sex	Days between injury and sample collection	Measured			
						Isoprostane	Histology	Glutathione	Acrolien
1	A–D myelomalacia	Chihuahua	2.5	m	3				X
2	A–D myelomalacia	Sheltie	1.5	f	1			X	
3	A–D myelomalacia	Mixed breed	5	f	2	X	X		
4	A–D myelomalacia	Dachshund	7	m	4	X	X	X	
5	A–D myelomalacia	Mini schnauzer	2.5	m	3	X			
6	Focal injury	Unknown	11.5	m	1				
7	Focal injury	Unknown	1.5	m	1	X			
8	Focal injury	Cocker spaniel	3	f	3	X	X	X	
9	Focal injury	Mixed breed	3	f	2	X			
10	Focal injury	Mixed breed	6	m	2	X			
11	Normal	Unknown	Unknown	Unknown	n/a		X	X	X
12	Normal	Mixed breed	4	f	n/a				
13	Normal	Shar pei	13	f	n/a	X			
14	Normal	Shar pei	2	f	n/a	X			
15	Normal (bladder infection)	Labrador retriever	6	m	n/a	X			
16	Normal	Mixed breed	12	m	n/a	X			
17	Normal	Unknown	Unknown	Unknown	n/a	X			
18	Normal	Unknown	Unknown	Unknown	n/a	X			
19	Normal	Unknown	Unknown	Unknown	n/a	X			
20	Normal	Unknown	Unknown	Unknown	n/a	X			