Naturally Occurring Disk Herniation in Dogs: An Opportunity for Pre-Clinical Spinal Cord Injury Research

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Abstract

Traumatic spinal cord injuries represent a significant source of morbidity in humans. Despite decades of research using experimental models of spinal cord injury to identify candidate therapeutics, there has been only limited progress toward translating beneficial findings to human spinal cord injury. Thoracolumbar intervertebral disk herniation is a naturally occurring disease that affects dogs and results in compressive/contusive spinal cord injury. Here we discuss aspects of this disease that are analogous to human spinal cord injury, including injury mechanisms, pathology, and metrics for determining outcomes. We address both the strengths and weaknesses of conducting pre-clinical research in these dogs, and include a review of studies that have utilized these animals to assess efficacy of candidate therapeutics. Finally, we consider a two-species approach to pre-clinical data acquisition, beginning with a reproducible model of spinal cord injury in the rodent as a tool for discovery with validation in pet dogs with intervertebral disk herniation.

Key words: animal model; canine; trauma

Introduction

AT LEAST 230,000 PEOPLE in the United States are living with disabilities related to spinal cord injury (SCI), with 10,000–12,000 people newly affected each year (Anonymous, 2010). These data highlight that although the incidence of SCI is low, affected patients live for prolonged periods of time, often with limited recovery of function. The disabilities associated with SCI impact quality-of-life metrics and worker productivity. Years lost to disability, a measure of the impact of disease on society, is greater in SCI than in the majority of traumatic events requiring hospitalization (Polinder et al., 2007). The cost of care delivery ranges from \$729,000–\$3,200,000 over an individual's lifetime (Anonymous, 2010).

The development of candidate therapeutics for SCI has been directed towards mitigating acute secondary injury (neuroprotection) (Baptiste and Fehlings, 2007), and facilitating regeneration and/or plasticity (Ruff et al., 2008). In the past decade, this effort has primarily relied on rodent models of SCI. These models produce stereotypical injuries, allow for uniform treatment intervals, and usually have a homogeneous genetic background. While these attributes enhance the potential detection of outcome associations, they do not nec-

essarily mimic the heterogeneity in timing and severity that occurs in humans with SCI (Tator, 2006). In 2007 Dobkin (Dobkin, 2007) estimated that >60 Phase II and Phase III clinical trials have been performed in humans with SCI, with only 1–2 trials showing potential enhanced recovery with experimental interventions. The reasons for failed therapeutic trials may relate to the way in which SCI is modeled and how pre-clinical data are obtained.

Canine thoracolumbar intervertebral disk herniation (IVDH) is a spontaneous disease that bears similarities to acute SCI in humans. Despite its high incidence, few pre-clinical trials have utilized dogs with IVDH (Table 1; Baltzer et al., 2008; Blight et al., 1991; Borgens et al., 1999; Jeffery et al., 2005; Laverty et al., 2004). A lack of familiarity with canine IVDH among researchers and a paucity of collaborative networks involving both the veterinary and basic science communities are likely to be contributing factors. Here we review the basic pathobiology, functional assessments, and outcomes associated with canine IVDH and its relevance to human SCI. We also consider a pathway for validation of an intervention prior to human clinical trials that begins with confirmation of efficacy in rodent models and subsequent validation in canine IVDH.

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TABLE 1. SUMMARY OF RECENT CLINICAL TRIALS INVOLVING DOGS WITH THORACOLUMBAR INTERVERTEBRAL DISK HERNIATION (IVDH)-ASSOCIATED SPINAL CORD INJURY (SCI)

<i>Clinical presentation</i>	<i>Design</i>	<i>Drug/control (number of dogs)</i>	<i>Drug target</i>	<i>Dosing strategy</i>	<i>Outcome metrics</i>	<i>Findings relative to controls</i>	<i>Reference</i>
IVDH T3-L3 Incomplete and complete SCI	Phase II Randomized, blinded controlled trial	N-acetylcysteine (35)/saline (35)	Reactive oxygen species	Initiated ≤ 7 days post-SCI; intravenous delivery over 60 min	Modified Frankel scoring	No improvement up to 42 days after SCI	Baltzer et al., 2008
IVDH and traumatic SCI Complete SCI	Phase I/II Open label No controls	Autologous olfactory glial cells (8)	Neurites/axons	Delivery via myelotomy 5 days to 10 months after SCI	Adapted Basso, Bresnahan, and Beattie rodent score for dogs	Improvement in limb movements in some dogs	Jeffery et al., 2005
IVDH T3-L3 Complete SCI	Phase II Open label Historical controls	Polyethylene glycol or poloxamer 188 + methylprednisolone sodium succinate(16)/historical controls (36)	Cell membrane	Initiated ≤ 3 days post SCI; intravenous delivery over 15 minutes with second dose 4-6 h later	Total neurological score (includes assessment of nociception, proprioception, somatosensory evoked potentials)	Improvement in score 6-8 weeks after SCI	Lavery et al., 2004
Majority were IVDH Complete SCI	Phase II Randomized, blinded controlled trial	Oscillating field stimulation (20)/sham implantation (14)	Neurites/axons	Device implanted within 18 days of SCI and activated 48-96 h after implantation	Total combined neurological score (includes spinal reflexes, ambulation, urethral pressures, somatosensory evoked potentials)	Improvement in score at 6 months after SCI	Borgens et al., 1999
Majority were IVDH T3-L3 Incomplete and complete SCI	Phase I/II Open label No controls	4-Aminopyridine (39)	Potassium channels	Intravenous delivery and oral 1-66 months after SCI	Weight bearing, balance, spinal reflexes, proprioceptive placing, nociception	Improvement in outcome metrics after drug delivery	Blight et al., 1991

Overview of Intervertebral Disk Herniation

Etiology

Thoracolumbar IVDH is a frequent cause of acute SCI in dogs and represents 2.3% of all admissions to veterinary teaching hospitals (Priester, 1976). The most commonly affected dogs are young to middle aged, male, chondrodystrophoid breeds (achondroplastic dwarf-type dogs such as Dachshunds, Pekingese, and Shih Tzus; Gage, 1975; Goggin et al., 1970; Priester, 1976). Epidemiological data indicate that 48–72% of affected dogs are Dachshunds, which have a lifetime incidence of IVDH that approaches 20% (Ball et al., 1982; Gage, 1975; Goggin et al., 1970; Priester, 1976). Dachshunds and other chondrodystrophoid breeds have early-onset disk degeneration typified by loss or dysfunction of notochordal and chondrocyte-like cells within the nucleus pulposus (Cappello et al., 2006; Hunter et al., 2004). The loss of these cells leads to metabolic disk degeneration, in which the nucleus pulposus becomes mineralized, dehydrated, and exhibits altered glycosaminoglycan content (Ghosh, 1990; Ghosh et al., 1977). Disk degeneration reduces the ability of the annulus fibrosus and nucleus pulposus to resist loading forces. Mechanical failure of a disk ensues, resulting in rupture of the annulus fibrosis and nuclear extrusion (extrusive IVDH or disk extrusion; Hansen, 1952). Data support a genetic basis for both disk degeneration and herniation in Dachshunds, although candidate genes have yet to be identified. Radiographically diagnosed disk degeneration in Dachshunds correlates with parentage, with a heritability estimate ranging from 0.46–0.87 (Jensen and Christensen, 2000). Pedigree analysis in Dachshunds has revealed an autosomal polygenic mode of inheritance for disk herniation (Ball et al., 1982).

Pathologic findings

Dogs with IVDH present with spinal cord compression and/or contusion (Griffiths, 1972; Hansen, 1952). Compressive material often contains extruded nucleus pulposus and hemorrhage which is located extradurally, ventral or ventrolateral to the spinal cord (Besalti et al., 2006; Hansen, 1952; Tartarelli et al., 2005). Hemorrhage originates from ventral vertebral venous sinuses that are damaged following extrusion of nuclear material (Tartarelli et al., 2005). Compression associated with IVDH may be focal or dispersed over several vertebrae (Besalti et al., 2006; Levine et al., 2009b). In rare instances, spinal cord laceration occurs due to bullet-like, low-volume disk extrusion that penetrates the meninges (Liptak et al., 2002; Sanders et al., 2002).

Lesions in dogs with IVDH are variable and suggest a mixed form of injury that preferentially involves ventral, lateral, or dorsolateral white matter (Fig. 1; Griffiths, 1972; Smith and Jeffery, 2006). Acute injuries produce intraparenchymal hemorrhage and edema (Smith and Jeffery, 2006). Axonal lesions are commonly observed and consist of early spheroid formation followed by axonal fragmentation and dissolution. One case series containing dogs with traumatic myelopathies and IVDH revealed spared, small diameter ($<5\mu\text{m}$) axons located around the periphery of the lesion epicenter (Smith and Jeffery, 2006). While some large-diameter axons appeared morphologically intact by light microscopy, subsequent ultrastructural evaluation revealed disruption of mitochondrial cristae (Smith and Jeffery, 2006).

Demyelination occurs within hours after canine IVDH-associated SCI (Smith and Jeffery, 2006; Summers et al., 1995). Myelin sheaths swell, degenerate, and then are removed by macrophages. Myelin loss usually occurs in concert with axonal degeneration, although paranodal demyelination with axonal preservation has been recognized (Smith and Jeffery, 2006; Summers et al., 1995). Gliosis involving astrocytes and microglia is evident in chronic lesions (Griffiths, 1972).

Grey matter lesions are often present in IVDH-associated SCI, and consist of hemorrhage, ischemic neuronal necrosis, and neuronal chromatolysis (Griffiths, 1972; Smith and Jeffery, 2006; Wright and Palmer, 1969). Ischemic neurons are typically replaced by microglial nodules. Vascular hyperplasia is common following IVDH-associated SCI, and is likely due to a combination of vascular reactivity and vascular proliferation (Griffiths, 1972; Smith and Jeffery, 2006; Summers et al., 1995; Wright and Palmer, 1969).

In severe IVDH-associated SCI, overt spinal cord necrosis has been recognized (Griffiths, 1972; Hoerlein, 1953; Smith and Jeffery, 2006; Wright and Palmer, 1969). These lesions vary in severity from focal areas of white matter destruction to gross myelomalacia that spans several spinal cord segments and involves the entire spinal cord cross-sectional area (Griffiths, 1972; Summers et al., 1995; Wright and Palmer, 1969). Focal areas of necrosis are circular to triangular, with the base of the triangle parallel to the pia mater, and resemble infarcts (Griffiths, 1972). Initially, these lesions appear pale and well-demarcated, and may contain neutrophils (Hoerlein, 1953; Smith and Jeffery, 2006). More chronic lesions consist of cavities/cysts that contain gitter cells, networks of blood vessels, and glial processes; chronic lesions are frequently surrounded by an increased number of astrocytes and microglia (Hoerlein, 1953; Smith and Jeffery, 2006). Typically, a thin rim of sub-pial white matter overlying necrotic spinal cord lesions is spared; this spared tissue has a putative role in functional recovery following SCI (Blight and Decrescito, 1986; Griffiths, 1972; Olby et al., 2003).

Comparative pathology

Gross and histopathologic lesions seen in canine IVDH resemble those recognized in some humans with traumatic myelopathies (Table 2). Solid (grossly normal spinal cord with lesions only visible on histopathology), contusion/cavity (normal spinal cord surface with gross evidence of intraparenchymal hemorrhage and necrosis with evolution to cysts), and massive compression (gross anatomic disruption and necrosis) lesions represent 79% of human SCIs (Norenberg and Smith, 2004); these patterns appear analogous to lesions identified in canine IVDH-associated SCI (Griffiths, 1972; Smith and Jeffery, 2006; Wright and Palmer, 1969). Axonal injury and associated demyelination are commonly identified in humans with SCI and dogs with IVDH (Kakulas, 1999; Norenberg and Smith, 2004; Smith and Jeffery, 2006; Wright and Palmer, 1969). Neuronal necrosis likewise occurs in both species (Kakulas, 1999; Norenberg and Smith, 2004; Smith and Jeffery, 2006; Wright and Palmer, 1969). Although the timing of inflammation following canine IVDH-associated SCI is not well described, the general pattern of early neutrophil infiltration followed by gitter cell predominance is analogous to what has been described in human SCI

FIG. 1. Hematoxylin and eosin stained spinal cord sections from dogs with thoracolumbar disk extrusion, showing the range of histopathologic lesions. Dog 1 (A) was paraplegic with preserved nociception for 6 h prior to sample acquisition. The spinal cord displays very mild white matter vacuolation (axonal degeneration and secondary demyelination) within the ventral and lateral funiculi (arrow). Dog 2 (B) was paraplegic without nociception for 2 days and has more extensive injury, including severe white matter vacuolation, intraparenchymal hemorrhage, and a discrete area of necrosis (asterisk). Dog 3 (C) was paraplegic without nociception for 14 days and also has extensive white matter vacuolation and a discrete area of necrosis that has become cavitated (asterisk). Dog 4 (D) was paraplegic without nociception for 3 months and had a discrete area of axonal loss and gliosis in the dorsal funiculi (arrow). Dog 5 (E) had paraplegia and absent nociception for 1 day. There is massive necrosis of both the white and gray matter of the spinal cord with intraparenchymal hemorrhage. Image F is a higher-magnification view of the discrete necrotic area in B. The necrotic, pale-staining area on the left (asterisk) contrasts with the still viable white matter on the right. Swollen axons (black arrow) and reactive blood vessels (white arrow) are evident. Image G is a closer view of the necrotic area in C. The necrotic area (asterisk) has become cavitated and contains numerous gitter cell macrophages (black arrow). Swollen axons (white arrow) are evident in the adjacent white matter. Image H is a higher-magnification view of the dorsal funiculi of image D, showing relatively normal white matter on the right side of the image with axonal loss and gliosis on the left.

TABLE 2. COMPARATIVE PATHOLOGIC AND IMAGING FEATURES OF HUMAN TRAUMATIC MYELOPATHY, CANINE THORACOLUMBAR IVDH-ASSOCIATED SPINAL CORD INJURY, AND RODENT EXPERIMENTAL CONTUSIVE SPINAL CORD INJURY

<i>Species/etiology</i>	<i>Primary injury</i>	<i>Injury level^{a,b}</i>	<i>Gross features^c</i>	<i>Acute histopathology^{c,d}</i>	<i>Chronic histopathology^{c,d,e}</i>	<i>Early MRI features^{f,g,h}</i>
Human traumatic spinal cord injury	Ventrally oriented compression contusion laceration	Cervical (61.2%) Thoracic (25.2%) Lumbosacral (13.6%)	Solid cord injury (10%) Contusion/cavity (49%) Laceration (21%) Massive compression (20%)	Inflammation Edema Hemorrhage Axonal pathology Demyelination Neuronal injury	Inflammation Wallerian degeneration Astroglial scar Cysts/cavities Syringohydromyelia Schwannosis	Compression T2W hyperintensity T2*W hypointensity T1W hyperintensity
Dog/IVDH-associated spinal cord injury	Ventrally oriented compression contusion	T10-L7 vertebral levels (83.6%)	Solid cord injury Contusion/cavity Massive compression Laceration was rare	Limited data; pathology appears similar to humans	Wallerian degeneration Cysts/cavities Spared peripheral white matter Data limited concerning presence of glial scar and other late features	Compression T2W hyperintensity T1W hyperintensity and T2* hypointensity have not been reported
Rat/traumatic spinal cord injury (contusion model)	Dorsally oriented weight-drop injury	Midthoracic	Contusion/cavity	Inflammation Edema Hemorrhage Axonal pathology Demyelination Neuronal injury	Inflammation Wallerian degeneration Demyelination Astroglial scar Cysts/cavities Schwannosis	T2W hyperintensity DWI hyperintensity

^aDistribution of human injuries taken from Varma et al., 2010; ^bDistribution of thoracolumbar IVDH-associated injuries from Gage, 1975; ^cData pertaining to human gross features from Norenberg and Smith, 2004; ^dRodent histopathology from Noble and Wrathall, 1989 and Beattie et al., 1997; ^eDog histopathology from Smith and Jeffery, 2006; ^fHuman MRI data from Miyajima et al., 2007; ^gDog MRI data from Levine et al., 2009b; ^hRat MRI data from Ellingson et al., 2010.

DWI, diffusion-weighted imaging; SC, spinal cord; T1W, T1-weighted; T2W, T2-weighted; T2*W, T2*-weighted; IVDH, intervertebral disk herniation; MRI, magnetic resonance imaging.

(Hoerlein, 1953; Norenberg and Smith, 2004; Smith and Jeffery, 2006). Cystic lesions at sites of necrosis and neovascularization have been identified in both species in the weeks and months after SCI (Norenberg and Smith, 2004; Smith and Jeffery, 2006).

Measuring Injury Severity

The severity of IVDH-associated SCI is variable and can be characterized clinically using behavioral data, magnetic resonance imaging (MRI), and electrophysiology.

TABLE 3. A COMPARISON OF COMMONLY USED ORDINAL PHYSICAL EXAMINATION-BASED SPINAL CORD INJURY SCALES ACROSS SPECIES

<i>American Spinal Cord Injury Association Impairment Scale (ASIA) (human)^a</i>	<i>Modified Frankel score (dog)^b</i>	<i>Texas Spinal Cord Injury Scale (TSCIS), gait (dog)^b</i>	<i>14 Point motor scale (dog)^c</i>	<i>Basso, Bresnahan, and Beattie (BBB) scale (rat)^d</i>
A=Complete; no motor/sensory function caudal to lesion	0=No motor or deep nociception caudal to lesion	0=No limb movement	0=No motor or deep nociception caudal to lesion	0=No limb movement
B=Incomplete; no motor function caudal to lesion; sensory preserved	1=No motor function or superficial nociception caudal to lesion; deep nociception preserved 2=No motor function caudal to lesion; all nociception preserved	0=No limb movement	1=No motor function caudal to lesion; deep nociception preserved	0=No limb movement
C=Incomplete; motor function caudal to lesion, but >50% of affected muscles lack movement against gravity; sensory preserved	3=Non-ambulatory with paresis and ataxia	1=Limb protraction with no ground clearance 2=Limb protraction with inconsistent ground clearance 3=Limb protraction with consistent ground clearance	3=Non-weight-bearing protraction in at least 1 joint 5=Non-weight-bearing protraction with >1 joint involved >50% of the time	1=Slight movement of 1–2 joints 3=Extensive movement of 2 joints 7=Extensive movement of all pelvic limb joints
D=Incomplete; motor function caudal to lesion and >50% of muscles have movement against gravity; majority ambulatory	4=Ambulatory with paresis and ataxia	4=Ambulatory, consistent ground clearance, moderate ataxia and paresis 5=Ambulatory, consistent ground clearance, does not fall	7=Weight-bearing protraction 10–50% of the time 10=Weight-bearing protraction 100% of the time with reduced strength; errors 50–90% of the time	10=Occasional weight-supported plantar steps without thoracic limb-pelvic limb coordination 13=Frequent to consistent weight-supported steps with limb coordination 17=Consistent plantar stepping/coordination; frequent toe clearance; predominant paw position parallel
E=Normal; possible hyperesthesia	5=Normal gait with possible hyperesthesia	6=Normal gait	14=Normal gait	21=Normal gait

^aVan Middendorp et al., 2009; ^bLevine et al. 2009a; ^cOlby et al., 2001; ^dBasso et al., 1995.

Neurologic assessments

Ordinal physical examination-based assessments, similar to those utilized in humans and rodents with SCI, have been developed and validated in dogs with naturally occurring myelopathies (Table 3). In 2001, Olby and colleagues (Olby et al., 2001) described a 14-point pelvic limb motor score for IVDH-associated SCI in dogs based on the Basso, Beattie, and Bresnahan (BBB) scale (Basso et al., 1995) used in rodents. More recently, a modified Frankel score and an assessment tool evaluating gait, postural reactions, and nociception (Texas Spinal Cord Injury Score) were described in dogs with SCI (Levine et al., 2009a). The modified Frankel score and Texas Spinal Cord Injury Score correlate well with one another, have strong inter-rater agreement, predict functional outcome in dogs with thoracolumbar IVDH, and are associated with spinal cord signal characteristics on MRI. Computed gait analysis may offer a more precise and objective means to characterize gait abnormalities in dogs with SCI. Recently, thoracic limb-pelvic limb coordination was quantified using digital motion capture in dogs with IVDH-associated SCI (Hamilton et al., 2007). In these animals, the pelvic limb step cycle was significantly slower than the thoracic limb step cycle, when compared to normal dogs and those with orthopedic disease. Kinematic gait analysis, similar to that described in rodents (Boyd et al., 2007), has also been per-

formed in dogs with thoracolumbar IVDH, using a pressure walkway with affected animals having longer pelvic limb stride length, stride time, and swing time compared to control dogs (Gordon-Evans et al., 2009).

MRI findings

As in humans with SCI, MRI offers a surrogate means to assess morphology and pathology in dogs with thoracolumbar IVDH. Extensive extradural compression on sagittal T2-weighted images, and the presence of intraparenchymal T2-weighted hyperintensity, are associated with severe SCI as determined by the modified Frankel score (Levine et al., 2009b). The presence of pre-surgical spinal cord T2-weighted hyperintensity is also negatively correlated with post-surgical functional recovery (Figs. 2 and 3; Ito et al., 2005; Levine et al., 2009b). In one report, the odds of long-term ambulation decreased by nearly twofold per unit (length of affected spinal cord:length of L2 vertebral body) of visualized intraparenchymal T2-weighted hyperintensity seen on sagittal images, independent of the modified Frankel score (Levine et al., 2009b).

Electrophysiology

Cortical and spinal somatosensory evoked potentials (SSEPs) have been used to evaluate spinal cord function in

FIG. 2. Sagittal (A) and transverse (B and C) T2-weighted magnetic resonance images from a dog with acute-onset non-ambulatory paraparesis. At the T13–L1 vertebral articulation there is severe ventral extradural spinal cord compression (A and C). The compressive material is non-dispersed and hypointense, which is common in some cases of disk extrusion that do not result in epidural hemorrhage. The spinal cord parenchyma cranial to the lesion (B), and at the lesion (A and C), has normal signal characteristics.

FIG. 3. Sagittal (A) and transverse (B and C) T2-weighted magnetic resonance images from a dog with acute-onset paraplegia and no deep nociception. At the L5 vertebra (A and C) there is dispersed, mixed-signal, ventral extradural compression due to disk extrusion and epidural hemorrhage. The spinal cord parenchyma cranial to the lesion (A and B) is hyperintense, suggesting severe spinal cord injury.

dogs with thoracolumbar IVDH (Borgens et al., 1999; Poncelet et al., 1998; Shores et al., 1987). These studies are performed by stimulating sensory nerve fibers in the pelvic limb and measuring far field potentials caudal, adjacent, and cranial to the site of SCI. Recorded spinal SSEPs may originate from the dorsal columns or dorsolateral fasciculus, whereas cortical SSEPs arise from electrical events in cortical neurons, synapses, or axons (Poncelet et al., 1998; Shores et al., 1987). Dogs with functional spinal cord transection have absent SSEPs cranial to a lesion, and injury potentials may be identified at the lesion site. If recovery occurs, SSEPs are recordable cranial to the lesion, although delays in latency may occur, possibly related to loss of myelin, or large-diameter, fast conducting axons (Borgens et al., 1999).

Treatment and Recovery

Dogs with significant neurological dysfunction due to IVDH are typically treated via decompressive laminectomy, which is often part of the treatment paradigm in humans with traumatic myelopathy. Voluntary ambulation occurs in 86–96% of dogs with IVDH that have pelvic limb nociception intact (based on behavioral assessment) prior to decompression (Davis and Brown, 2002; Ferreira et al., 2002; Necas, 1999; Ruddle et al., 2006). Only 43–62% of dogs with IVDH that have absent responses to pelvic limb deep nociceptive stimulation will ambulate following decompressive surgery (Duvall et al., 1996; Ito et al., 2005; Olby et al., 2003). Paraplegic dogs with IVDH that have intact nociception pre-surgically

have a median time to regain ambulatory function following decompression of 9 days (Ferreira et al., 2002). In dogs lacking pre-surgical deep nociception, the mean time to ambulation is 7.5 weeks (Olby et al., 2003). Ordinal assessment scores may continue to improve, even in ambulatory dogs, for at least 12 weeks following injury (Olby et al., 2004). The rapidity of neurological deterioration, MRI signal of the spinal cord, and concentration of myelin basic protein (MBP) in the cerebrospinal fluid (CSF), all appear to influence post-surgical outcome beyond traditional nociceptive assessment (Levine et al., 2010, 2009b; Scott and McKee, 1999). While CSF MBP may be a prognostic indicator in humans with head trauma, it has not been investigated in humans with acute SCI (Berger et al., 2006; Mukherjee et al., 1985).

IVDH: Strengths and Strategies for Overcoming Its Limitations

Naturally occurring canine thoracolumbar IVDH has many features that make it suitable as a model for human SCI. Perhaps one of the most elemental advantages of canine IVDH compared to experimental models is that the SCI is spontaneous. Dogs with IVDH are not anesthetized when SCI occurs and therefore are not exposed to neuroprotection that may result from anesthesia-associated changes in body temperature and CNS metabolism (Park et al., 2005; Sang et al., 2006). Medical management is similar to that in humans. As in humans, there is a delay between SCI and assessment at a medical facility; analgesics are administered following SCI;

and anesthesia, decompressive surgery, and physical rehabilitation are part of standard treatment (Griffin et al., 2009; Olby, 1999; Olby et al., 2005). The relative age and gender of dogs with IVDH-associated SCI are very similar to those of humans with SCI; namely, affected animals are typically young adult males. Dogs with thoracolumbar IVDH are also likely more genetically diverse than inbred rodent strains, which can have significant strain-to-strain variation in response to SCI (Steward et al., 1999).

Importantly, the mechanisms and pathology underlying canine IVDH-associated SCI are similar to those in human traumatic myelopathies. The primary injury mechanisms involve both compression and contusion and lesion severity is variable, which mimics the situation in clinically-affected humans (Griffiths, 1972; Hansen, 1952; Smith and Jeffery, 2006; Tator, 2006). Spinal cord signal characteristics on T2-weighted MRI are associated with outcome and injury severity, as is the case in people with SCI (Levine et al., 2009b; Miyajima et al., 2007; Ramon et al., 1997). The larger physical size of the canine spinal cord compared to that of the rodent may more closely mimic the challenges associated with axonal sprouting across glial scars seen in humans (Jeffery et al., 2006). Finally, the size of dogs also facilitates locoregional delivery of cellular therapeutics, acquisition of significant quantities of CSF, and serial blood withdrawal for pharmacokinetic studies.

Surrogate markers for injury severity can be studied via *in vivo* evaluation of CSF. For example, matrix metalloproteinase-9, which is upregulated in the acutely injured spinal cord and a determinant of long-term outcome in rodents, has been detected in the CSF of dogs with IVDH resulting in paraplegia (Levine et al., 2006; Noble et al., 2002). This protease is likewise elevated in CSF from human patients with multiple sclerosis, and is correlated with periods of exacerbation of the disease (Leppert et al., 1998; Muroski et al., 2008). MBP has also been detected in the CSF of dogs with a <7-day history of thoracolumbar IVDH, and was recently shown to be an independent prognostic biomarker in this population (Levine et al., 2010). Higher CSF MBP concentrations were associated with poorer long-term functional outcome, perhaps due to an immune-mediated response to MBP, or because MBP concentration correlated with the extent of spinal cord demyelination. Cartilage oligomeric matrix protein is normally found in canine, mouse, and rat spinal cord grey matter extracellular matrix (Tokunaga et al., 2010). It has been detected in the CSF of dogs with thoracolumbar IVDH, although correlations with injury severity or outcome have not been established (Tokunaga et al., 2010).

Finally, the study of canine IVDH-associated SCI is feasible. Incidence of the disease is high, resulting in robust caseloads at academic centers that have well-developed treatment programs (Gage, 1975; Goggin et al., 1970; Priester, 1976). Clients are typically willing to participate in trials or non-invasive research studies. In fact, our current case accrual rate for clinical trial participation approximates 90%. Also, the study of naturally occurring disease offers a rare win-win scenario in which both humans and affected dogs stand to benefit.

Strategies for overcoming limitations

There are shortcomings associated with IVDH as a pre-clinical model. Injury is spontaneous and therefore cases are

not admitted at preset time intervals. There is variability in the time from injury to treatment, injury severity, and the contributions of primary and secondary SCI mechanisms. Methylprednisolone sodium succinate is not a uniformly accepted standard of care (Boag and Drobatz, 2001; Olby, 1999). This may be an advantage of canine IVDH from a discovery standpoint, but data obtained in dogs that are not administered methylprednisolone may not be applicable to humans that receive this treatment. As in human trials, histopathology is not a viable means to determine outcome in dogs with IVDH, and a proportion of dogs in clinical trials are lost to follow-up. This has typically ranged from 15–50% of enrollees in most completed and ongoing canine SCI trials (Baltzer et al., 2008; Borgens et al., 1999; Laverty et al., 2004). Additionally, the fiscal cost of performing canine SCI trials is substantially greater than rodent studies. At our institution, the diagnosis and treatment (surgical decompression and rehabilitation) of canine thoracolumbar IVDH currently costs between \$3500 and \$5000. Considering that most dogs with IVDH weigh between 5 and 20 kg, the cost of novel pharmaceutical therapeutics is also substantially greater than in rodents.

Finally, there are anatomical and physiological differences between the dog and human spinal cord. For example, the corticospinal tracts are more important in ambulatory function in humans compared to dogs (Hukuda et al., 1973). Likewise, the relatively small canine corticospinal tract may explain why spinal shock is more transient in dogs with IVDH compared to humans with SCI (Smith and Jeffery, 2005). The vertebral level at which human traumatic myelopathies occur is diverse. In one report 61.2% of paralyzed individuals had cervical lesions and 38.8% had trauma to the thoracic or lumbar vertebral column; cervical lesions are of particular concern, as they result in the highest degree of injury-associated morbidity and mortality (Varma et al., 2010). In dogs with thoracolumbar IVDH, 83.6% of injuries are located between T11 and L3 (Gage, 1975). Also, while aspects of lesion pathology appear similar between dogs with IVDH and humans with traumatic SCI, a large body of comparative data is not currently available.

Adhering to human SCI clinical trial principles can minimize limitations that pertain to injury timing, injury severity, and subject accrual. Since dogs with IVDH are client owned, data concerning the safety of a therapeutic must be present before initiating a study on treatment efficacy. Once sufficient safety data are available, a phase II study is usually constructed. Phase II studies are performed at a single center, contain 20–200 patients, and examine a limited number of outcome measures. They ideally should be randomized placebo-controlled trials (RCTs; Lammertse et al., 2007). Strict inclusion criteria are formulated to limit the study population to dogs most likely to respond to an intervention. Dogs that have received certain pre-referral treatments, such as glucocorticoids, can be excluded if appropriate. The time between injury and intervention is recorded, and dogs can be stratified during data analysis based on this parameter. Similarly, initial ordinal neurological score and MRI signal characteristics can be used to subclassify SCI severity. Recovery has usually been measured via ordinal neurological scores recorded daily during post-injury hospitalization, and then at a standardized re-check interval (typically 4–12 weeks following SCI; Baltzer et al., 2008; Borgens et al., 1999; Olby et al., 2004). Serial

electrophysiology, measures of urinary bladder voiding, kinematic and kinetic gait analysis, proxy quality-of-life scores, and MRI may also be valid outcomes to examine (Borgens et al., 1999; Hamilton et al., 2007; Levine et al., 2008). Phase III studies always recruit patients from multiple centers, follow an RCT design, and derive protocols and outcome measures from phase II data showing therapeutic efficacy (Lammertse et al., 2007). There are no completed phase III canine SCI trials to date, although a study comparing polyethylene glycol, methylprednisolone, and saline placebo in dogs with IVDH is ongoing.

Completed Canine SCI Clinical Trials

A limited number of clinical trials have been performed in dogs with naturally occurring thoracolumbar SCI resulting from IVDH. Most studies have been phase I/II designs, but significant variation exists between reports with regard to follow-up intervals, assessed outcomes, use of blinding or randomization, means of adverse event description, and pre-study biological data to support trial protocols. Clinical trials using dogs with IVDH have assessed both neuroprotective and regenerative/plasticity strategies.

Neuroprotective trials

Laverty and colleagues (Laverty et al., 2004) have examined the effect of polyethylene glycol (PEG) on motor and sensory recovery in dogs with thoracolumbar IVDH that lacked pelvic limb deep nociception. The basis for this study was data from SCI in guinea pigs showing conduction of evoked potentials through the lesion, recovery of the cutaneous trunci reflex, and sparing of spinal cord parenchyma following PEG delivery (Borgens, 2001; Borgens and Shi, 2000; Borgens et al., 2002; Duerstock and Borgens, 2002). These effects are putatively mediated through PEG's ability to penetrate CNS tissue and act as a surfactant, which may result in fusion of transected axons and sealing of disrupted myelin (Borgens, 2001). Administration of PEG to dogs in the study group resulted in improved sensory and motor recovery compared to control animals of matched injury severity. The control group, however, was non-contemporaneous and selected from medical records (historical controls), which may have been a source of selection bias. The investigation was also an open-label investigation, and thus subject to the placebo effect that commonly enhances outcomes in non-blinded clinical studies. Finally, recent investigations performed by independent laboratories (Ditor et al., 2007; Kwon et al., 2009) have not clearly demonstrated improved histological or motor outcomes following delivery of PEG alone in rat models of SCI. Despite these limitations, data concerning PEG are promising and have prompted an ongoing phase III trial in dogs with IVDH, and a planned human SCI clinical trial examining PEG plus magnesium.

A recent clinical trial (Baltzer et al., 2008) investigated N-acetylcysteine (NAC) as a neuroprotective therapy in dogs with thoracolumbar IVDH with and without pelvic limb deep nociception. This intervention was examined due to the presence of markers of oxidative stress in the urine of dogs with IVDH, known antioxidant properties of NAC, the wide safety margin of NAC across species, and data from experimental SCI models that indicated enhanced motor recovery with NAC (Cakir et al., 2003; McMichael et al., 2006). The

study was a phase I/II design, and importantly incorporated blinding and randomization. Motor recovery was not enhanced in dogs given NAC. This may be due to the small number of dogs in the study (study power was not calculated *a priori*), variable timing between injury and inclusion, variable injury severity, and use of a very abbreviated neurological score (3 grades), which likely limited the ability to detect subtle outcome differences between groups. As adverse events were not associated with NAC, and the study may not have been designed adequately to detect outcome associations, further investigation of this therapeutic may be warranted.

Regeneration/plasticity trials

Blight and colleagues (Blight et al., 1991) investigated the effects of 4-aminopyridine (4AP) on dogs with traumatic and IVDH-associated SCI. The majority of animals in the study had an absence of pelvic limb deep nociception. Data to support this intervention included the identification of conduction block in spared myelinated axons from cats with experimentally-induced SCI, and *in vitro* evidence to suggest that 4AP enhances conduction in injured axons (Blight, 1983, 1989). In cats with experimental SCI, 4AP was also shown to enhance vestibulospinal reflexes (Blight and Gruner, 1987). In 64% of dogs studied, 4AP administration resulted in rapid but transient improvements in pelvic limb placing and/or nociception. Adverse events were uncommon, but occasionally severe. Outcome data from this report must be viewed cautiously, as it was designed to assess the safety of 4AP in dogs with SCI (phase I study). There was no control group and the study was open label. Several human clinical trials occurred subsequent to the study of 4AP in dogs with naturally occurring SCI. In some human clinical trials, administration of sustained-release 4AP enhanced motor and sensory function (Potter et al., 1998); in other studies benefits have been modest or non-existent (Cardenas et al., 2007; DeForge et al., 2004).

Oscillating field stimulation (OFS) has been examined in dogs with thoracolumbar IVDH that lacked pelvic limb deep nociception (Borgens et al., 1999). Prior to that study, OFS had been shown to enhance axonal regeneration and reduce axonal degeneration across several SCI models. Data had also been obtained in dogs with IVDH that suggested improvement across a limited number of outcomes using an early OFS technique (Borgens et al., 1993). The study by Borgens and colleagues that was performed in 1999 was a RCT phase II trial utilizing an improved OFS device (Borgens et al., 1999). Animals in the treatment group had significantly higher combined neurologic scores (behavioral assessments of motor and sensory function, electrophysiology, and urodynamics) compared to sham-treated dogs. Certain patient populations, such as older animals and those with lower motor neuron signs, were excluded from the study, which may not make findings applicable to all groups with SCI. A human phase I trial using OFS for SCI has been performed; the device appeared safe, and improvements were seen in select sensory and motor outcomes (Shapiro et al., 2005).

Autologous olfactory ensheathing cells (OECs) have been studied in dogs with severe, chronic, naturally occurring thoracolumbar SCI resulting from IVDH and exogenous trauma (Jeffery et al., 2005). This therapeutic was investigated due to evidence from rodent SCI models that suggests the

OECs enhance axonal sprouting across lesions and improve motor outcomes (Keyvan-Fouladi et al., 2003; Li et al., 2003a, 2003b). The study was designed to assess whether harvest of OECs from dogs was feasible and OECs could be expanded *in vitro*. Additionally phase I data were obtained concerning the delivery of OECs via midline myelotomy to dogs with chronic SCI. No significant adverse events appeared to be related to OEC implantation. In addition, the majority of dogs had improved motor function over the months (range 2–12 months) following delivery; only 1 of 8 studied dogs regained pelvic limb nociception. Since the study was a phase I design primarily performed to assess feasibility of this intervention, no control group was utilized; thus results concerning outcomes must be interpreted cautiously. The lack of pelvic limb nociception in the majority of animals may indicate that motor recovery represented local reflex (“spinal”) walking, and was not due to axons traversing the lesion site (Jeffery et al., 2005). Currently a phase II clinical trial using an RCT design is being performed using OECs in dogs with severe naturally occurring SCI.

A Proposed Two-Species Model for Pre-Clinical Studies

In experimental models of SCI, efficacy of a candidate therapeutic is rarely validated in a second species. As such it is difficult to know if a therapeutic benefit can be generalized to other species including humans. Additionally, the majority of published rodent experimental studies concerning outcome effects of novel interventions have not been externally validated. The National Institute of Neurological Disorders and Stroke (NINDS) has attempted to address this issue through the “Facilities of Research–Spinal Cord Injury” (FOR-SCI) project, which is focused on the independent replication of published studies. The value of FOR-SCI and similar endeavors has been demonstrated in recent reports (Aguilar and Steward, 2010; Steward et al., 2008). For example, replication of an experiment that had previously established positive outcome effects associated with Nogo-66 receptor antagonism in mice generated dissimilar findings (Steward et al., 2008). Here we consider a paradigm whereby a therapeutic is studied in a rodent model of SCI, and then further validated in dogs with IVDH prior to the initiation of human clinical trials. The objectives of this approach are to limit false discovery, enhance the quality of pre-clinical data, and improve the risk-reward profile of human phase II and phase III studies.

Rodent models of SCI are characterized by their reproducibility, and as such are a suitable platform for biological discovery and testing efficacy of a therapeutic (Kwon et al., 2010). In cases for which a promising therapeutic has been reported in a peer-reviewed journal, we support its further validation in dogs with naturally occurring IVDH. The benefits of the IVDH model, in particular similarities to facets of human SCI, can be borne out in well-designed, randomized controlled phase II studies (Kwon et al., 2010). This multi-tiered paradigm has the potential to enhance discovery and improve the validation of therapeutics prior to human clinical trials. By placing the emphasis on stronger pre-clinical data, human clinical trials will be limited to the therapeutics with the most potential. Enhanced pre-clinical data will also improve estimates of population size that are essential in appropriate human phase II studies; underpowered phase II studies are common, and can

lead to a failure to detect clinically important outcome differences between groups (type II error).

Conclusions

Canine IVDH has similarities to human SCI with regard to age, gender, pathophysiology, MRI features, surgical and rehabilitative treatments, and outcomes. As such it is a suitable complementary platform for validating therapeutics that have been shown to be efficacious in traditional rodent models of SCI. Unfortunately, canine IVDH is underutilized in this respect. A PubMed search (search dates January 1, 1950–August 12, 2010) of the terms “dog disk herniation” or “dog disk disease” revealed only 211 citations, with most identified manuscripts focused wholly on veterinary clinical applications. While there are likely to be a number of factors that contribute to the scarcity of these studies, including cost, there is also a lack of cross-disciplinary synergy between basic scientists, physicians, and veterinarians. Bridging that gap is a prerequisite step toward building a testing paradigm that applies carefully-validated rodent studies to rigorously-designed pre-clinical studies in dogs with IVDH.

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