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Cerebrospinal fluid tau protein as a biomarker for severity of spinal cord injury in dogs with intervertebral disc herniation

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ABSTRACT

Intervertebral disc herniation (IVDH) is a common cause of spinal cord injury (SCI) in dogs. Microtubule-associated protein tau derives predominantly from neurons and axons, making it a potential marker of neuronal injury. A retrospective study, including 51 dogs with thoracolumbar or cervical IVDH and 12 clinically normal dogs, was designed to describe associations between cerebrospinal fluid (CSF) tau concentration, degree of neurological signs and motor functional recovery in dogs with IVDH. Signalment, degree of neurological dysfunction and outcome were recorded. Cisternal CSF tau values were determined by ELISA. Associations between CSF tau concentration and various clinical parameters were evaluated. Receiver-operating characteristics curve (ROC) analyses were performed to assess the validity of protein tau measurements.

CSF tau concentrations were significantly higher in dogs showing plegia (median, 79.9 pg/mL; range, 0–778.7 pg/mL; $P = 0.016$) compared to healthy dogs and dogs with paresis (median, 30.1 pg/mL; range, 0–193.1 pg/mL; $P = 0.025$). Plegic dogs that improved by one neurological grade within 1 week had significantly lower tau protein levels compared to plegic dogs that needed more time for recovery or did not show an improvement ($P = 0.008$). A CSF tau concentration >41.3 pg/mL had a sensitivity of 86% and specificity of 83% to predict an unsuccessful outcome in plegic dogs based on ROC analysis (area under the curve, 0.887; $P = 0.007$, 95% confidence interval [CI] 0.717–1.057). CSF protein tau levels are positively associated with the severity of spinal cord damage and may serve as a prognostic indicator in dogs with IVDH.

Introduction

Tau proteins belong to the microtubule-associated proteins family (Weingarten et al., 1975). These phosphoproteins specifically localize in neurons where they bind to microtubules, promoting their assembly and stability (Weingarten et al., 1975; Binder et al., 1985; Brandt and Lee, 1993; Wang and Liu, 2008). Tau is not physiologically secreted and its release into the cerebrospinal fluid (CSF) is most probably due to neuron damage or death (Mori et al., 1995). Therefore, CSF tau protein concentrations can serve as a biological marker for axonal damage in the central nervous system (CNS) (Zemlan et al., 1999; Shiiya et al., 2004).

In humans, tau is encoded by a single gene that undergoes alternate splicing resulting in six tau isoforms which range from 352 to 441 amino acids (Goedert et al., 1989). Tau proteins can be separated into two groups, namely, the low molecular weight tau proteins expressed in neurons of the CNS, and the high molecular weight tau proteins most abundant in the peripheral nervous system (Georgieff et al., 1993). Tau proteins play an important role in the neuronal microtubules network involved in axonal transport and neuronal transmission (Drubin, 1986). By regulating microtubule assembly, tau proteins participate in modulating axonal morphology and growth (Buee et al., 2000).

Elevated levels of CSF tau protein have been described in human neurodegenerative disorders such as Alzheimer's disease and multiple sclerosis (Blennow et al., 1995; Andreasen et al., 1998; Terzi et al., 2007). Information on tau protein in veterinary medicine is currently rare and so far only tau protein expression in the aging brains of dogs and cats, as well as CSF tau levels in dogs with encephalitis, have been examined (Head et al., 2005; Pugliese et al., 2006; Tanaka et al., 2011).

Intervertebral disc herniation (IVDH) is a common cause of spinal cord injury (SCI) in dogs, resulting in pain and neurological dysfunction. Severity of neurological signs is determined by neuroanatomical location, velocity and amount of the compressive material as well as duration of compression (Brisson, 2010). IVDH leads to SCI via a combination of primary and secondary events. Primary spinal cord injury refers to the initial mechanical insult, whereas secondary injury is a biochemical cascade following the primary event and consisting of vascular dysregulation, neurogenic shock, oxidative stress and excitotoxicity (Dumont et al., 2001).

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Several prognostic factors for IVDH were previously studied in CSF samples, such as the percentage of CSF macrophages (Srugo et al., 2011), CSF levels of myelin basic protein and creatinine kinase activity (Levine et al., 2010; Witsberger et al., 2012), concentration of beta-2-microglobulin (Muñana et al., 2007) and CSF glutamate concentration (Olby et al., 1999). These studies included dogs with thoracolumbar IVDH or dogs with acute signs (Levine et al., 2006, 2010; Srugo et al., 2011). Therefore, additional reliable prognostic factors that allow for differentiation between good and poor functional outcome are needed. Moreover, clear cut-off points for unfavourable outcome should be stated (Levine et al., 2010; Witsberger et al., 2012).

CSF tau levels are highly elevated in human head trauma patients and a correlation has been established between clinical improvement and decreased CSF tau levels (Zemlan et al., 1999). We therefore hypothesized that high levels of protein tau reflect the severity of spinal cord damage and that determination of CSF tau concentration has a value as a prognostic biomarker for motor functional recovery in dogs with IVDH.

Material and methods

Study design and animals

Medical records of the Department of Small Animal Medicine and Surgery, University of Veterinary Medicine, Hannover, were reviewed for dogs diagnosed with IVDH (2005–2011). The study was performed according to the ethical rules of the University.

On the basis of the severity of the neurological signs, 51 dogs were categorized according to Sharp and Wheeler (2005) into five grades: paraspinal hyperesthesia (grade 1); ambulatory paresis, ataxia and proprioceptive deficits (grade 2); non-ambulatory paresis (grade 3); plegia with nociception (grade 4) and plegia with no deep nociception (grade 5). Inclusion criteria for this study were dogs suffering from cervical or thoracolumbar IVDH (Hansen type I and II) as suggested by magnetic resonance imaging (MRI) findings and in most cases confirmed by decompressive surgery, a severity of grade 2–5 at presentation and subsequent analysis of CSF. Dogs were re-examined neurologically 7 days after the first presentation.

The information retrieved from the medical records included signalment (sex, age, breed), time from onset of clinical signs to presentation, previous drug administration with emphasis on glucocorticosteroids, localization of the disc protrusion/extrusion, the occurrence of pathological changes in the myelon detected by magnetic resonance imaging (MRI) and functional neurological outcome (Levine et al., 2009; Boekhoff et al., 2012). All MR images were reviewed by certified neurologists for the presence of an intramedullary hyperintensity over the length of one vertebral body or more (Levine et al., 2009). The outcome was defined to be successful if the dog improved by at least one neurological grade within a week after the first presentation at the Department of Small Animal Medicine and Surgery.

Normal research colony dogs ($n = 8$; Animal Experiment number 33.42502/05-12.05) as well as four dogs presented at our clinic because of orthopaedic, non-neurologic diseases, were used as a control population. All control dogs were required to have normal physical and neurological examinations as well as CSF analysis.

Cerebrospinal fluid collection

Cerebrospinal fluid (CSF) was collected at the time of presentation/admission under general anaesthesia from the cerebellomedullary cistern. Samples (200 μL) without iatrogenic blood contamination were subsequently used for routine examination (cell count, glucose and protein concentrations) and the remaining CSF samples were frozen and stored in polypropylene tubes at −20 °C (Lachno et al., 2011).

Biochemical analysis of tau

Tau protein was determined by a commercially available enzyme-linked immunosorbent assay (ELISA) (Innotest hTAU, Innogenetics), which recognizes non-phosphorylated and phosphorylated tau (Vandermeeren et al., 1993). It is a solid-phase enzyme immunoassay in which tau protein or tau fragments are captured by a primary monoclonal antibody (AT120) and two biotinylated secondary antibodies (HT7bio and BT2bio). Fifty microlitres of CSF were used for each measurement, each sample was measured in duplicates, and the mean was used for further evaluation. Previous examinations excluded interferences in the assay format for haemoglobin, bilirubin, albumin or globulin (Nishimura et al., 1998). Preliminary studies defined a detection limit of 18.7 pg/mL.

Statistical analysis

Data were analysed using a commercially available software package (GraphPad Prism Version 5.0, GraphPad Software). Variables were controlled for normal distribution using Kolmogorov–Smirnov normality test. As data were not consistent with a Gaussian distribution, non-parametric tests (Kruskal–Wallis and Mann–Whitney U test) were applied. All statistical tests were two-tailed and $P \leqslant 0.05$ was considered to be statistically significant.

For the purpose of analysing the relation between tau protein levels and severity of neurological dysfunction, dogs were divided into two groups (A: paresis = grade 2/3; B: plegia with and without deep pain sensation = grade 4/5). Within these groups dogs were further categorized based on occurrence of functional improvement by one grade within 7 days or no functional improvement.

For the purpose of describing duration of clinical signs before admission dogs were divided into three subcategories (acute, <5 days; subacute, 5–14 days; chronic, >14 days). Dogs were divided in two groups for analysing the effect of administration of corticosteroids (yes vs. no), location of the spinal cord compression (cervical vs. thoracolumbar) and spinal cord TW2 hyperintensity (yes vs. no).

Receiver-operating characteristics (ROC) curve analysis was performed to determine the overall effectiveness of CSF tau concentration to predict an unsuccessful outcome in dogs showing plegia due to IVDH. Additionally, ROC curve analysis was used to find out if CSF tau concentrations are able to distinguish between paretic and plegic dogs. The cut-off that maximized the Youden index (sensitivity + specificity − 1) was selected as optimal.

Results

Fifty-one dogs with cervical ($n = 18$) or thoracolumbar ($n = 33$) IVDH of various breeds and with a different degree of neurological dysfunction met the inclusion criteria. The mean age of the dogs was 7.3 years (range, 3–12.25 years). Breeds included Dachshund ($n = 19$), mixed breed ($n = 13$), Beagle ($n = 2$), Bernese Mountain dog ($n = 2$), Cocker Spaniel ($n = 2$), Rottweiler ($n = 2$), French Bulldog ($n = 2$) and nine other breeds with one dog each. Twenty-three male, 11 castrated male, 7 female and 10 spayed female dogs were examined. The neurological examination revealed 24 dogs with paresis and/or ataxia with proprioceptive deficits (grade 2), whereas 7 dogs were non-ambulatory paraparetic (grade 3). Fourteen dogs displayed plegia with nociception (grade 4), whereas in six dogs nociception was lost (grade 5). Dogs with paresis (grade2/3, $n = 31$) and dogs showing plegia (grade 4/5, $n = 20$) were combined for further evaluation. Forty-four dogs (grade 2: $n = 18$; grade 3: $n = 7$; grade 4: $n = 14$; grade 5: $n = 5$) underwent decompressive surgery whereas six dogs (all classified as grade 2) were treated conservatively and one dog (grade 5) was euthanased after diagnostic imaging. Glucocorticosteroids were administered to 23/51 dogs with IVDH (45.1%) before presentation (Table 1).

The control group consisted of 12 dogs with a mean age of 5.0 years (range, 1.8–8.8 years). One female, two spayed females, two males and seven castrated males were examined. Breeds included Beagle ($n = 8$) and one of each of the following breeds (mixed breed, Labrador Retriever, Boxer, Chihuahua).

Tau was measurable in 7/12 dogs of the control group (58.3%) and in 33/51 dogs with IVDH (64.7%). Median tau protein level in the control group was 20.6 pg/mL (range, 0–51.2 pg/mL), whereas in dogs with IVDH it was 41.8 pg/mL (range, 0–778.7 pg/mL). Significant differences ($P = 0.049$) were detected between CSF tau concentrations in dogs with IVDH and dogs of the control group. These differences were even more distinct with evaluation of neurological grade (Fig. 1). CSF tau concentrations were significantly ($P = 0.016$) higher in dogs with plegia (grade 4/5; median, 79.9 pg/mL; range, 0–778.7 pg/mL) compared to control dogs. CSF tau levels were not significantly different between dogs with paresis (grade 2/3; median, 30.1 pg/mL; range, 0–193.1 pg/mL) and control dogs (median, 20.6 pg/mL; range, 0–51.2 pg/mL). In dogs with IVDH, plegic dogs showed significantly higher tau concentrations than paretic dogs ($P = 0.025$).

A significant association was found between CSF tau concentration and functional outcome in dogs affected by IVDH. Dogs with IVDH ($n = 51$) that improved by one neurologic grade within a