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CHANGES IN THE CREATINE KINASE AND LACTATE DEHYDROGENASE ACTIVITIES IN CEREBROSPINAL FLUID OF DOGS WITH THORACOLUMBAR DISC DISEASE

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Abstract

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Ninety-seven dogs (49 males and 48 females) with thoracolumbar (TL) disc disease which were treated at the Clinic of Surgery and Orthopedics at University of Veterinary and Pharmaceutical Sciences in Brno from October 1997 till the end of December 1998 were included in this study. A total of 142 cerebrospinal fluid (CSF) samples were evaluated. CSF from the cisterna magna (cisterna cerebellomedularis) was collected successfully in all 97 animals. Only 45 samples were obtained from lumbar subarachnoid space in the same patients. The purpose of our study was to determine whether transverse myelopathy due to TL disc protrusion/extrusion may cause elevation of CSF creatine kinase (CK) and lactate dehydrogenase (LDH) activities in dogs. Normal values of CSF CK (0.41 ± 0.43 μkat/l) and LDH (0.40 ± 0.28 μkat/l) activities were determined in 23 healthy dogs. The highest normal enzyme activity was assessed as $s_{\Delta 0.95}$ (mean + 2 SD). Activity of CSF CK 1.27 μkat/l, and activity of CSF LDH 0.96 μkat/l were considered to be abnormal. Significant differences (Mann-Whitney *U*-test; $p < 0.01$) between activities of the two enzymes in the cerebrospinal fluid of healthy dogs and dogs with TL disc extrusion (the mean CSF CK = 2.47 ± 3.22 μkat/l; the mean CSF LDH = 1.45 ± 1.98 μkat/l) were found. CSF from lumbar and atlantooccipital punctures in 45 patients, in which both the samples were successfuly collected, were analyzed and compared statistically. The mean CK activity of the lumbar CSF samples was 2.47 ± 3.22 μkat/l; the mean CK concentration of atlantooccipital CSF was 0.50 ± 0.47 μkat/l. The mean of lumbar CSF LDH activities was 1.45 ± 1.98 μkat/l; the mean of atlantooccipital CSF LDH values was 0.34 ± 0.32 μkat/l. Statistical analysis (Wilcoxon matched-pairs signed-ranks test) indicated that differences in the CK and LDH concentrations between CSF samples obtained from the cisterna magna, and from lumbar subarachnoid space were significant ($p < 0.01$). The dynamics of CSF CK and LDH activities in dogs with disc disease was assessed, as well. The mean of CSF CK activity taken on the first day of paraparesis/paraplegia was 1.47 ± 1.22 μkat/l. Creatine kinase in the cerebrospinal fluid had peaked at 48 hours after onset of clinical signs (3.84 ± 4.95 μkat/l). On the third and fourth days its activity had significantly decreased ($p < 0.01$) to 1.76 ± 2.71 μkat/l. A second peak was identified ($p < 0.05$) between day seven and seventeen (3.80 ± 3.23 μkat/l). The mean LDH concentration in the CSF on day one was 1.32 ± 1.28 μkat/l, but its concentration increased significantly ($p < 0.01$) during the second day of clinical signs (2.36 ± 3.38 μkat/l). Mean LDH concentration decreased significantly ($p < 0.01$) on the third and the fourth days (0.80 ± 0.33 μkat/l) without a second peak of activity.

Cerebrospinal fluid, creatine kinase, lactate dehydrogenase, dynamics, disc disease, prognosis, dog

Little has been published on the use of cerebrospinal fluid enzyme studies in dogs, in respect to intervertebral disc disease (IVDD) (Indrieri et al. 1980; Wilson 1977; Wright 1980). Numbers of dogs with IVDD in these previous clinical studies were limited. Experimental findings concerning the CSF enzyme levels in dogs after asphyxiation and cardiac arrest have been documented (Vaagenes et al. 1988; Vaagenes et al. 1997). It is generally accepted that cerebrospinal fluid reflects the metabolism of the brain and spinal

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cord (Lamers and Wevers 1995). Lesions of tissues rich in enzymes, corresponding with their damage or structural destruction, may cause enzymatic release. Increased enzyme levels in cerebrospinal fluid (CSF), including creatine kinase (CK) and lactate dehydrogenase (LDH), have been reported to indicate tissue damage of the central nervous system (CNS) or altered permeability of barriers (Mayhew and Beal 1980). Elevated levels of the enzymes in CSF are usually not associated with elevated serum enzyme levels in dogs (Mayhew and Beal 1980) and in people (Šafář et al. 1982). No significant correlation between CK activities of CSF and plasma in cattle was found (Buchner et al. 1996), as well. These enzyme tests are not specifically diagnostic but can aid in the distinction between structural and functional disease of CNS (Mayhew and Beal 1980). To our knowledge, dynamics of the CSF enzyme changes in dogs with compressive myelopathy secondary to intervertebral disc lesions have not been previously documented in veterinary literature. Valuable information concerning CSF CK activity relative to duration and severity of clinical signs is available in the human medical literature (Šafář et al. 1982). Klun (1974) reported that in human patients with CNS trauma, CSF enzyme values corresponded to the clinical severity of the injury, with the highest values occurring two to nine days after injury, and enzyme elevations proportional to the extent of tissue damage. Culebras-Fernández (1971) stated that diseases chronically affecting gray matter and acute, extensive diseases of CNS may cause elevation in the LDH levels, whereas diseases affecting the myelin may cause elevated values of CK in people. In veterinary medicine the CSF CK activity was evaluated in dogs (Abate et al. 1998; Indrieri et al. 1980; Wilson 1977; Wright 1980) and horses (Furr and Tyler 1990; Jackson et al. 1996). Creatine kinase activity was increased in cases of feline toxoplasmosis and feline infectious peritonitis (Wilson 1977).

Contrary to the enzyme activity changes, increases in CSF protein concentration, which may develop in association with most CNS diseases are well documented (Thomson et al. 1990). This study stressed the importance of CSF collection from the appropriate site for accurate documentation of changes secondary to focal CNS disease. CSF collected caudal to the lesion is more consistently abnormal than CSF sample obtained cranial to the lesion (Bailey and Higgins 1985). Thomson et al. (1990) believe that is because of predominant caudal flow of CSF. The order in which CSF is collected from the lumbar subarachnoid space or cisterna magna does not affect the values obtained in healthy dogs (Bailey and Higgins 1985; Duncan et al. 1987), however, it may be important in dogs with focal neurologic lesion. The pressure gradient within the subarachnoid space may cause flow of CSF toward the site of fluid withdrawal (Thomson et al. 1990).

The aim of this experimental study

The purpose of our study was to determine whether transverse myelopathy due to TL disc protrusion/extrusion may cause elevation of CSF creatine kinase and lactate dehydrogenase activities in dogs. Our goal was to assess dynamics of CSF CK and LDH activities in dogs with intervertebral disc disease. The reason was to prove that actual enzyme concentration depends on the duration of clinical signs at the time of cerebrospinal fluid collection. The differences between the CSF CK and LDH concentrations in samples obtained by atlantooccipital and lumbar punctures, respectively, were evaluated.

Materials and Methods

Ninety-seven dogs with TL intervertebral disc disease (IVDD) treated at the Clinic of Surgery and Orthopedics at University of Veterinary and Pharmaceutical Sciences in Brno from October 1997 till the end of December 1998 were included in this study. In each case, the diagnosis of TL - IVDD was confirmed by clinical evaluation, radiography and surgery. Dogs (both male and female) with compressive myelopathy caused by TL disc protrusion/extrusion were candidates for cerebrospinal fluid analysis. All dogs were classified into groups according to severity of signs (Toombs and Bauer 1993). Dogs with grade I, grade II and grade IV C clinical

signs were excluded from this study. Dogs with an initial episode of back pain and no neurological deficits (grade I) are treated conservatively and CSF samples are seldom collected from these patients. Patients with grade II paraparesis were excluded because of the small number of cases (n = 2). No dogs with grade IV C paraplegia (duration of deep pain absence more than 48 hours) were presented to our hospital during the period of this clinical study.

Cerebrospinal fluid was obtained from dogs that were under general anesthesia prior to myelographic examination. The general anesthesia protocol used in a given patient was dependent upon its general condition. Patients without a risk of anesthesia according to ASA classification (Paddleford and Erhardt 1992) were premedicated with 30-50 µg/kg of medetomidine (Domitor®, Pfizer GmbH, Germany) given IM. General anesthesia was induced with propofol (Diprivan®, Zeneca Limited, Great Britain) 2-4 mg/kg IV to effect. Endotracheal intubation was performed and a surgical plane of anesthesia was maintained by administration of a mixture of oxygen and halothane (Narcotan®, Léčiva, Czech Republic) (1.0-2.5%). Patients at potential risk (according to ASA classification) were intravenously premedicated with combination of 0.5-0.8 mg/kg droperidol and 10-16 µg/kg fentanyl (Thalamonal®, Janssen Pharmaceutica, Belgium). General anesthesia was induced with 2-4 mg/kg propofol given IV to effect. Isoflurane (Forane®, Abbott Laboratories Ltd., Great Britain) (1.0-2.5%) was used instead of halothane for maintenance inhalation anesthesia. Antibiotics (cefazolin 22 mg/kg IV; Kefzol®, Eli Lilly Italia S.p.A., Italy), steroids (methylprednisolone sodium succinate 30 mg/kg IV slowly in infusion; Solu-Medrol®, Upjohn s.a., Puurs, Belgium) and ranitidine (1-2 mg/kg; Ulcosan®, Galena, Czech Republic) were given perioperatively.

The CSF collection site was clipped and aseptically prepared. CSF was collected from the cisterna magna and by lumbar transmedullary puncture from the ventral subarachnoid space between L5-6 vertebrae using 22G spinal needle (Spinocan®, B. Braun Melsungen AG, Germany). The atlantooccipital puncture was made before the lumbar puncture and new spinal needle was used for each site. The stylet of the needle was removed after atlantooccipital membrane and dura mater penetration, and after accessing ventral part of subarachnoid space in lumbar area, respectively. The lumbar subarachnoid space was punctured under fluoroscopic control. Samples from lumbar puncture were not assayed in cases, where an appropriate volume of cerebrospinal fluid could not be obtained, or when the sample was macroscopically contaminated by blood. The net effect of these problems was that fewer lumbar aspirates were available for analysis.

Fluid for CSF analysis was analyzed within 30 minutes of collection. The total creatine kinase and lactate dehydrogenase activities of each sample were examined using automated chemistry analyzer Cobas Mira S (Roche Diagnostic Systems, Inc.). Cerebrospinal fluid CK activity was measured by spectrophotometric method using diagnostic kit "CK-NAC®" (BioVendor, Czech Republic). This method is based on the original formulation of Oliver optimized by Szasz. CK catalyzes the reaction of creatine phosphate with ADP. ATP produced in this reaction is measured by coupling the hexokinase and the GPD (glucose-6-phosphate-dehydrogenase) reaction to the CK reaction. The rate of NADPH formation at 340 nm is a measure of the CK activity, provided that the concentrations of all other components in system are present in suitable excess, so that the CK activity is the only limiting factor. Measurement of LDH activity was done with commercially available diagnostic kit "LDH®" (BioVendor, Czech Republic). In this analysis pyruvate is reduced to lactate at a pH of 7.4 and a temperature of 37 °C. The progress of accompanying oxidation of NADH to NAD^+ is monitored continuously by measuring the rate of absorbance decrease at 340 nm. Enzyme activity was expressed as µkat/l.

Normal values of CSF CK and LDH activities were determined in cerebrospinal fluid samples from the lumbar subarachnoid space of 23 healthy dogs in which CSF was collected for other purposes.

Means and standard deviations were calculated for all variables. If normal distribution of measured values was not found, the values were statistically analyzed using an Mann-Whitney *U*-test, and Wilcoxon matched-pairs signed-ranks test, respectively. In cases of normal distribution of values in data sets a Student's *t*-test was used. Statistical analyses were done using Stat plus 1.10 (Matoušková et al. 1992).

Results

A total of 142 samples from 97 dogs (49 males and 48 females) with TL - IVDD were evaluated. CSF from the cisterna magna was collected successfully in all 97 animals. Only 45 samples were successfully collected from lumbar subarachnoid space. Breeds represented were Dachshund (73.20%; n = 71), Pekingese (7.22%; n = 7), mixed-breed (7.22%; n = 7), American Cocker Spaniel (3.09%; n = 3), Poodle (2.06%; n = 2), Basset Hound (2.06; n = 2), Shih Tzu (1.03%; n = 1), Lhasa Apso (1.03%; n = 1), French Bulldog (1.03%; n = 1), Cocker Spaniel (1.03%; n = 1) and Miniature Schnauzer (1.03%; n = 1). The age of the dogs varied from 2 to 11 years with an average of 6.01 ± 2.10 years.

In clinically healthy dogs (12 males and 11 females) creatine kinase activities ranged from 0 to 1.51 µkat/l, with the average of 0.41 ± 0.43 µkat/l. The mean value and standard deviation for activity of the lactate dehydrogenase in the CSF of normal dogs was

0.40 ± 0.28 µkat/l. Individual values varied from 0.10 to 1.18 µkat/l. The age in normal dogs ranged from 1.5 to 18 years, with the average of 5.65 ± 3.66 years. Breeds represented in this group were German Shepherd (30.43%; n = 7), Dachshund (21.73%; n = 5), mixed-breed (13.04%; n = 3), Dobermann (8.70%; n = 2), Schnauzer (8.70%; n = 2), Poodle (4.35%;
n = 1), Shar-pei (4.35%; n = 1), French Bulldog (4.35%; n = 1) and Spitz (4.35%; n = 1).

Table 1
The CK and LDH activities (mean ± SD) in the CSF of healthy dogs, and patients with thoracolumbar intervertebral disc disease (IVDD), collected from the cisterna magna and the lumbar subarachnoid space, respectively

Group of dogs	**Number of samples** n=165	**CK (µkat/l)** mean ± SD	**LDH (µkat/l)** mean ± SD
Healthy dogs	23	0.41 ± 0.43	0.40 ± 0.28
Dogs with IVDD Atlantooccipital puncture**	97	0.57 ± 0.58	0.33 ± 0.30
Dogs with IVDD Lumbar puncture***	45	2.47 ± 3.22	1.45 ± 1.98

** Samples obtained from the cisterna magna
*** Samples collected from the lumbar subarachnoid space

Table 1 shows mean concentrations of CK and LDH in the CSF of healthy dogs and in CSF samples obtained both from the cisterna magna and from the lumbar subarachnoid space in patients with TL - IVDD. Statistically significant differences between CSF CK and LDH activities in healthy dogs and TL – IVDD patients (samples punctured from lumbar SA space) were found using Student's *t*-test ($p < 0.01$).

Using the Mann-Whitney *U*-test, significant differences ($p < 0.01$) between CSF CK and LDH activities in 97 samples collected from the cisterna magna and 45 samples from the lumbar subarachnoid space were found. Enzyme concentrations were higher in samples obtained by lumbar puncture (Table 1). This result was confirmed using the Wilcoxon matched-pairs signed-ranks test ($p < 0.01$) to analyze paired values in the 45 dogs (Table 2) for which both atlantooccipital and lumbar CSF samples were available.

Table 2
The CK and LDH activities (mean ± SD) in the CSF of patients with thoracolumbar intervertebral disc disease (IVDD), collected from the cisterna magna and the lumbar subarachnoid space, respectively

Dogs with IVDD	**Number of samples** n=90	**CK (µkat/l)** mean ± SD	**LDH (µkat/l)** mean ± SD
Atlantooccipital puncture**	45	0.50 ± 0.47	0.34 ± 0.32
Lumbar puncture***	45	2.47 ± 3.22	1.45 ± 1.98

** Samples obtained from the cisterna magna
*** Samples collected from the lumbar subarachnoid space

The mean and standard deviation for activities of CSF CK and LDH in samples collected from the cisterna magna of dogs within different clinical groups (grade III, IV A, IV B) are shown in Table 3. Similar data for cerebrospinal fluid samples obtained from the lumbar subarachnoid space are listed in Table 4. A higher concentration of the CSF LDH in dogs

Table 3
The CK and LDH activities (mean ± SD) in the cerebrospinal fluid collected from the cisterna magna in 97 dogs with IVDD

Severity of clinical signs*	**Number of samples** n=97	**CK (µkat/l)** mean ± SD	**LDH (µkat/l)** mean ± SD
Grade III	23	0.62 ± 0.53	0.29 ± 0.19
Grade IV A	47	0.48 ± 0.57	0.35 ± 0.34
Grade IV B	25	0.69 ± 0.66	0.36 ± 0.33

* Classification of dogs into groups according to severity of clinical signs

Table 4
The CK and LDH activities (mean ± SD) in the cerebrospinal fluid collected from lumbar subarachnoid space in 45 dogs with IVDD

Severity of clinical signs*	**Number of samples** n=45	**CK (µkat/l)** mean ± SD	**LDH (µkat/l)** mean ± SD
Grade III	12	2.69 ± 2.50	1.00 ± 0.89
Grade IV A	22	2.15 ± 2.24	1.11 ± 0.61
Grade IV B	11	2.86 ± 5.27	2.63 ± 3.70

* Classification of dogs into groups according to severity of clinical signs

Table 5
Dynamics of changes in the CSF CK and LDH activities (mean ± SD) in samples obtained from lumbar subarachnoid space in 45 dogs with disc disease

Duration of clinical signs at the time of puncture Days	**Number of samples** n=45	**CK (µkat/l)** mean ± sd	**LDH (µkat/l)** mean ± sd
1	18	1.47 ± 1.22	1.32 ± 1.28
2	10	3.84 ± 4.95	2.36 ± 3.38
3 and 4	8	1.76 ± 2.71	0.80 ± 0.33
7 to 17*	9	3.80 ± 3.23	0.92 ± 0.64

* Samples from day 5 and 6 were not available

with grade IV B paraplegia compared to the other groups is evident (Table 4).

Differences in the activities of creatine kinase and lactate dehydrogenase in CSF obtained from the lumbar subarachnoid space in 45 dogs with TL - IVDD, related to duration of clinical signs at the moment of sample collection, are listed in Table 5. Normality in distribution of the measured values was not found. All data were analyzed using the Mann-Whitney *U*-test. The mean of CSF CK activity taken on the first day of paraparesis/paraplegia was 1.47 ± 1.22 µkat/l. Second day creatine kinase activity in the cerebrospinal fluid had increased significantly ($p < 0.01$) to 3.84 ± 4.95 µkat/l. Compared with the second day value, the CSF CK activity on the third and fourth days had significantly decreased ($p < 0.01$) to 1.76 ± 2.71 µkat/l. From the data available from the seventh day till the seventeenth day after onset of clinical signs it is obvious, that total CK activity in the

CSF had increased significantly ($p < 0.05$) for a second time (3.80 ± 3.23 μkat/l). The mean LDH concentration in the CSF on day one was 1.32 ± 1.28 μkat/l. Significantly increased ($p < 0.01$) LDH activities were observed by the second day of clinical signs (2.36 ± 3.38 μkat/l). The mean of the CSF LDH concentrations had decreased significantly ($p < 0.01$) on the third and the fourth days of paraparesis/paraplegia (0.80 ± 0.33 μkat/l). Compared with the second day value, the CSF LDH concentrations stayed significantly decreased ($p < 0.01$) from seventh till the seventeenth days after onset of clinical signs (0.92 ± 0.64 μkat/l). No data on day five and six were available.

Discussion

The anesthesia protocol for a given patient was selected on the basis of published data (Paddleford and Erhardt 1992; Thurmon et al. 1996; Wooten and Lowrie 1993) and our own experiences obtained during surgical treatment of TL - IVDD cases.

Controversy exists in available literature concerning possible contamination of the CSF sample during its collection. Although previous canine studies showed that CSF parameters differ depending upon the collection site, the high frequency of contamination of lumbar puncture CSF samples with blood remains a confounding variable (Bailey and Higgins 1985). Contamination of CSF samples by blood was found in nine of fourteen cases even though this was not grossly evident at the time of cisternal collection (Wright 1980). In CSF samples containing blood, CK values were increased whereas protein values were not (Indrieri et al. 1980). Hurtt and Smith (1997) examined the effects of iatrogenic blood contamination on total protein concentration and nucleated cell count in CSF from normal dogs and dogs with neurologic disease. They found that high CSF nucleated cell counts and protein concentrations are indicative of neurologic disease, even if samples contain moderate amounts of blood contamination. Jackson et al. (1996) proved that contamination of CSF with whole blood, hemolyzed red blood cells, or serum did not substantially contribute to increases in CSF CK activity. However, they found that addition of epidural fat or dura to CSF significantly increased creatine kinase activity in the cerebrospinal fluid in the horse. Based on these findings they postulated that the use of CSF CK activity as a diagnostic indicator of neurological diseases in horses is unreliable. We attempted to eliminate falsely increased CK activities due to contamination of CSF with epidural fat or dura during the fluid collection by our collection technique (maintaining the stylet within the spinal needle during its insertion into the subarachnoid space).

When selecting suitable methods of chemistry analysis for our experimental study, it was necessary to consider their availability regardless of the time of day, ease of laboratory technique, and price of the assay. The total creatine kinase and lactate dehydrogenase activities were examined on each fluid sample using automated chemistry analyzer. Each sample was evaluated within 30 minutes after collection to achieve maximal possible reliability of the fluid analysis. The CSF CK and LDH isoenzyme profiles were not analyzed because it was not our goal to establish the exact origin of the enzymes. We assumed that total enzyme values can display pathological changes in both nervous tissue and blood-brain barrier. Some authors report that increases in CK activity are associated with damage to white matter and myelin degeneration, as well as nerve cell body disruption (Furr and Tyler 1989; Hayes 1987). Others speculate that high creatine kinase activity results from blood contamination of the fluid sample due to changes in permeability of the blood-brain barrier (Indrieri et al. 1980; Wilson 1977). Abate et al. (1998) postulated that during neurological diseases, these enzymes are released in CSF as a consequence of tissue damage and/or increased permeability of the blood brain barrier. Heavner et al. (1986) found approximately 10 times higher activity of the LDH in serum, than in the CSF. The CSF LDH

isoenzyme profile was a mirror image of the serum lactate dehydrogenase isoenzyme profile. Simultaneous measurement of serum and cerebrospinal fluid LDH activity and LDH isoenzyme profile can aid in establishing status of the blood-brain barrier (Heavner et al. 1986). Creatine kinase brain isoenzyme (CK BB) is the only CK isoenzyme contained in neural tissue. It is an intracellular enzyme released in various neurologic conditions, including meningitis (Nussinovitch et al. 1996). Several techniques for the separation and assay of creatine kinase isoenzymes are available (Halonen et al. 1982; Wevers et al. 1984). The CK-BB isoenzyme determination is well documented in human patients with intracranial tumors and acute cerebral infarctions (Matias-Guiu et al. 1986), in hypoxic brain injury after cardiac arrest (Kärkelä et al. 1992), as well as in neonates with neurologic disorders (De Praeter et al. 1991). The brain isoenzyme efficacy is obvious in diagnosis of brain insult (Osuna et al. 1992), because CK-BB activity is an early indicator of brain damage and its level may reflect the extent of cerebral damage (Anagnostopoulos et al. 1988). An early biochemical indicator of irreversible changes could be helpful in surgical cases requiring prompt intervention (Chaloupka et al. 1997). The CK-BB isoenzyme determination is useful in predicting the outcome of patients with acute neurological symptoms as well (Bödvarsson et al. 1990). On the other hand, the total creatine kinase activity in the CSF in differential diagnosis of metabolic and organic causes of coma in man was measured (Galindo et al. 1995). The serum activity of creatine kinase was found to be independent of the CK activity in the cerebrospinal fluid (Šafář et al. 1982), so for purposes of our study there was no reason to distinguish both possible sources of the enzyme. Our goal was to prove increased activities of the CSF enzymes in dogs with TL - IVDD and determine dynamics of changes in enzyme concentrations.

Wilson and Wiltrout (1976) measured CSF CK levels in forty-four healthy dogs. In our study, the highest normal enzyme activity was assessed as $s_{\Delta 0.95}$. Activity of CSF CK 1.27 μkat/l (mean + 2 SD), and activity of CSF LDH 0.96 μkat/l (mean + 2 SD) were considered to be abnormal.

Indrieri et al. (1980) found increased CK activities in two (16.6%) of twelve dogs with intervertebral disc disease. Both samples were from dogs with a disc protrusion at C3-4 site. In another study (Wilson 1977) only one of three dogs with IVDD had increased creatine kinase activity in the CSF. Wright (1980) evaluated CSF in 7 dogs with thoracolumbar and 2 dogs with cervical IVDD. CSF CK activity was not increased in 11 horses with cervical compressive myelopathy, in 2 horses with extradural compressive tumors and one horse with disc protrusion in samples obtained at the lumbosacral site (Furr and Tyler 1990). In our study significant differences (Student's *t*-test; $p < 0.01$) between CSF CK and LDH activities in healthy dogs and clinical patients with TL – IVDD were found (Table 1). Our findings (Table 3) suggest that CK and LDH activities in the CSF collected from the cisterna magna (cranial to the lesion) may be normal or mildly altered by TL compressive myelopathy. Fluid obtained by lumbar puncture (caudal to the lesion) is more substantially altered (Table 4), because of the predominant caudal flow of CSF. In practice it is very important to obtain the fluid caudal to the lesion. Spinal cord diseases cannot be diagnosed by suboccipital puncture (Thomson et al. 1990). In animals with TL - IVDD it is important to colllect a lumbar CSF sample. It does not preclude the use of cisternal puncture to obtain a second CSF sample for comparison (Indrieri et al. 1980). Comparison of evaluated parameters was the reason why we collected both CSF samples. We did not evaluate the enzyme in serum, because the concentration of creatine kinase in CSF is independent of CK concentration in serum (Indrieri et al. 1980; Šafář et al. 1982).

In our 45 patients with IVDD, in which both cisternal and lumbar samples were successfully obtained, CSF samples collected from lumbar subarachnoid space were abnormal in 46.67% of the cases for both CK and LDH activities, compared with the highest

normal enzyme activity (mean + 2 SD) in our study. Great variability in the measured values was found. Although these CSF enzymes may be helpful in establishing of severity of the CNS damage due to a compressive lesion, the number of values which exceeded $s_{\Delta 0.95}$ (46.67%) was relatively small. This may be related to the time related dynamics of changes in the enzymes activity (all measured values are included in the analysis without relation to the time of sample collection), or to different degrees of damage to CNS tissues. The same criteria were fulfilled for cisternal samples in only 10.31% cases for CK concentrations, and 6.19% cases for LDH concentrations, respectively. This is because of the significantly lower enzyme activity typical of CSF obtained cranial to the lesion.

The concentration of creatine kinase in the cerebrospinal fluid may be of some prognostic value (Indrieri et al. 1980). Further investigations are necessary to prove or disprove the theory about the prognostic value of CSF CK activity because many clinical factors, including method of surgical treatment (Nečas 1995a), may play a role in outcome of surgical treatment of TL - IVDD in dogs, especially those with grade IV A and IV B paraplegia (Nečas 1995b).

Although the half-life of CSF CK is not known in dogs, CSF CK peaks at 12 hours after acute trauma and decreases to the normal value within 36 hours in people (Rabow et al. 1986); CK half-life was calculated to be approximately 10 hours. Šafář et al. (1982) found that serum CK activity peaks at 24 hours and CSF CK activity reaches its maximum at 48 hours in people with local cerebral ischemia. The two activities were found to be independent of each other. In our study the dynamics of changes in the CSF CK and LDH activities in dogs with TL - IVDD was assessed. The mean of CSF CK activity taken on the first day of paraparesis/paraplegia was 1.47 ± 1.22 μkat/l. Creatine kinase in the cerebrospinal fluid had peaked at 48 hours after onset of clinical signs (3.84 ± 4.95 μkat/l). On the third and fourth days its activity had significantly decreased ($p < 0.01$) to 1.76 ± 2.71 μkat/l. A second peak of activity was identified ($p < 0.05$) between day seven and seventeen (3.80 ± 3.23 μkat/l). The mean LDH concentration in the CSF on day one was 1.32 ± 1.28 μkat/l. Its concentrations had significantly increased ($p < 0.01$) by the second day of clinical signs (2.36 ± 3.38 μkat/l), and had decreased significantly ($p < 0.01$) by the third and the fourth days (0.80 ± 0.33 μkat/l) without a second peak of its activity. Further investigations in this area are desirable.

Změny aktivity kreatinkinázy a laktátdehydrogenázy v mozkomíšním moku u psů s onemocněním torakolumbálních meziobratlových plotének

Ve studii je zahrnuto 97 pacientů (49 psů a 48 fen) léčených s onemocněním torakolumbálních meziobratlových plotének na Klinice chirurgie a ortopedie VFU v Brně od října 1997 do konce prosince 1998. Vyšetřeno bylo celkem 142 vzorků mozkomíšního moku. U všech 97 zvířat byl úspěšně odebrán mok z cerebellomedulární cisterny. Z lumbálního subarachnoidálního prostoru se od těchto pacientů podařilo odebrat pouze 45 vzorků moku. Cílem naší studie bylo zjistit, zda příčná myelopatie v důsledku protruze/extruze disku v torakolumbálním úseku páteře u psů může být příčinou zvýšení aktivity kreatinkinázy (CK) a laktátdehydrogenázy (LDH) v mozkomíšním moku. Hodnoty normální aktivity CK (0.41 ± 0.43 μkat/l) a LDH (0.40 ± 0.28 μkat/l) v mozkomíšním moku jsme stanovili u 23 zdravých psů. Nejvyšší normální aktivitu těchto enzymů jsme určili jako $s_{\Delta 0.95}$ (aritmetický průměr + 2¥ směrodatná odchylka). Za abnormální jsme považovali aktivitu CK 1.27 μkat/l a aktivitu LDH 0.96 μkat/l. V případě obou enzymů jsme zjistili statisticky významný rozdíl (Mann-Whitney *U*-test; $p < 0.01$) mezi jejich aktivitou v mozkomíšním moku zdravých psů a pacientů s extruzí torakolumbálního disku (aktivita CK = 2.47 ± 3.22 μkat/l; aktivita LDH = 1.45 ± 1.98 μkat/l). Vzorky mozkomíšních moků od 45 psů, u nichž se mok podařilo odebrat jak lumbální, tak atlantookcipitální punkcí, jsme

analyzovali a statisticky porovnali. Průměrná aktivita CK ve vzorcích mozkomíšního moku odebraných lumbální punkcí byla 2.47 ± 3.22 μkat/l; průměrná koncentrace CK ve vzorcích odebraných atlantookcipitálně byla 0.50 ± 0.47 μkat/l. Průměrná hodnota aktivity LDH v mozkomíšním moku z lumbální punkce byla 1.45 ± 1.98 μkat/l; ve vzorku odebraném atlantookcipitální punkcí 0.34 ± 0.32 μkat/l. Statistická analýza (Wilcoxonův pořadový test) ukázala signifikantní rozdíly v koncentracích CK a LDH mezi vzorky mozkomíšního moku odebraného z atlantookcipitální cisterny a z lumbálního subarachnoidálního prostoru
($p < 0.01$). Rovněž byla zhodnocena dynamika aktivity CK a LDH v mozkomíšním moku u psů s onemocněním disků. Průměrná aktivita CK ve vzorcích odebraných první den po vzniku paraparézy/paraplegie byla 1.47 ± 1.22 μkat/l. Koncentrace kreatinkinázy v mozkomíšním moku dosáhla vrcholu za 48 hodin po vzniku klinických příznaků (3.84 ± 4.95 μkat/l). Třetí a čtvrtý den její aktivita signifikantně klesla ($p < 0.01$) na 1.76 ± 2.71 μkat/l. Druhý vrchol aktivity CK jsme prokázali ($p < 0.05$) mezi sedmým a sedmnáctým dnem (3.80 ± 3.23 μkat/l). Průměrná koncentrace LDH v mozkomíšním moku první den po vzniku klinických příznaků byla 1.32 ± 1.28 μkat/l. Její koncentrace signifikantně vzrostla ($p < 0.01$) druhý den (2.36 ± 3.38 μkat/l), a poklesla ($p < 0.01$) mezi třetím a čtvrtým dnem paraparézy/paraplegie (0.80 ± 0.33 μkat/l) bez dosažení druhého vrcholu v aktivitě tohoto enzymu.

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