Cerebrospinal fluid assessment in dogs with spinal tumors and intervertebral disk herniation

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Abstract

The study presents a retrospective case study. This aim was to determine whether lactate, glucose and total protein in serum and cerebrospinal fluid (CSF) can be used as specific biochemical markers for rapid differential diagnosis between intervertebral disk herniation and spinal tumors. Canine blood (n=9) and cerebrospinal fluid (n=9) were collected for chemistry analysis in nine dogs divided into two groups: intervertebral disk herniation group (IVDHG) (n=6) and spinal tumor group (TG) (n=3). Absorption spectrophotometry was used for quantitative determination of the concentrations of lactate, glucose and total protein in serum and CSF. Lactate concentration in serum and CSF and glucose concentration in CSF were 40-60% higher in subjects in TG compared to the IVDHG group. Serum lactate concentrations in the IVDHG group were 60% lower than physiological values. The results indicate that elevated concentration of glucose, lactate and total proteins in CSF and serum are indicative of a tumor, while a decrease of serum lactate and other parameters within normal range indicate disk herniation.

Key words: *dog; cerebrospinal fluid; spinal tumor; intervertebral disk herniation*

Introduction

Cerebrospinal fluid (CSF) is an aquatic solution within the subarachnoid space, the central canal of the spinal cord, and the four ventricles of the brain. The major constituent of cerebrospinal fluid is water (99%), whereas the remaining 1% consists of ions, proteins, glucose, lactose, and other organic compounds and cells (Khasawneh et al., 2018).

In current veterinary medicine, CSF serves as an invaluable diagnostic tool in the evaluation of inflammatory,

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infectious or non-infectious diseases, as well as in CT-negative subarachnoid hemorrhage and in leptomeningeal metastases (Deisenhammer et. al., 2011). Alterations of chemical composition (*i.e.* determination of protein, albumin, immunoglobulin, glucose, lactate, cellular changes, presence of specific antigen and antibodies) are associated with different pathological processes, so combining a set of CSF variables allows good diagnostic sensitivity and specificity.

The chemical composition of CSF varies following injury, trauma and/or compression, depending on the rate of development of the injury, its severity, location, time between the injury and collection of CSF, and the maintenance or progression of the injury. Acute trauma can occur with disk herniation and is always accompanied with inflammation and hemorrhage. An acute process, whether accompanied by another process or not, is easily seen from CSF composition. In spinal cord injury, the composition of lumbar CSF is changed more so than the cerebellomedullary CSF. Unlike acute diseases, chronic disease tends to produce milder changes. Tumor diseases also alter the chemical composition. CSF changes associated with neoplasia depend on the type of tumor, location and tissue reactions to the disease. The most common abnormality in tumor diseases is increased protein concentration, and therefore their concentrations are an important marker for the disease.

The aim of this study was to find determine the differences in the chemical composition of CSF and serum in patients with tumor and disk hernia. To do so, we tracked three parameters in serum and CSF, concentration of total proteins, glucose and lactate in dogs with tumor and disk herniation. The tumor group consisted of dogs with osteosarcoma, as the most common primary bone neoplasia in dogs (Belda et al., 2008).

Materials and methods

Use of archived samples of serum and CSF was ethically approved by the Ethics Committee at the Faculty of Veterinary Medicine, University of Zagreb (No. 640-01/18-17/48; 251-61-21/333-18-02).

Animals

A total of nine dogs of both sexes, mean age 6.38±2.44 years, were included in the current study. Dogs were divided into two groups according to disease. Canine blood (n=9) and cerebrospinal fluid (n=9) were collected for chemistry analysis in all nine dogs, divided into two groups: intervertebral disk herniation group (IVDHG) (n=6) and an spinal tumor group (TG) (n=3). Due to the biomechanics of the canine spine, intervertebral disk herniation most often occurs at the transition between the thoracic and lumbar spine. In this study, dogs with disk herniation at T11-12, T12-13, T13-L1 were included. All tumors were detected in the thoracolumbar part of vertebra and histologically evaluated as osteosarcoma. All dogs underwent a full physical examination before general anesthesia.

Anesthesia and sampling

Dogs in both groups were fasted for approximately 6 to 12 h before anesthesia. Propofol (Propofol 1%, Abbott, UK) was administered into the *vena cephalica antebrachii* until the onset of anesthesia. Anesthesia was maintained with propofol in a duration of two to five minutes during sampling of CSF, and the contrast was introduced into the subarachnoid space. CSF were taken by lumbar puncture and serum from *v. cephalica antebrachii.*

Biochemical analysis

Biochemical analysis of serum and CSF was performed by the light absorption spectrophotometry (VetTest, Idexx, USA) method. Due to instrumental limi-

tations (>1 g/L), protein values measured in the CSF of both groups was not analyzed statistically. Glucose, total proteins and lactate were measured in serum and CSF in both groups. Descriptive statistical methods were used; only average value and standard deviation are given.

Results

A total of nine samples of serum and CSF obtained from nine dogs with spinal tumor and intervertebral disk hernia were evaluated. CSF from the lumbar subarachnoid space was collected successfully in all animals. Breeds represented were Dachshund (*n*=2), Pekingese (*n*=1), mixed-breed (*n*=2), Doberman (*n*=2), Fox Terrier (*n*=1), and Rottweiler (*n*=1). The age of dogs varied from 4 to 11 years, with an average of 6.38±2.44 years. The ages of dogs with intervertebral disk herniation group (IVDHG) (*n*=6) ranged from 4-7 years old, while all dogs in the spinal tumor group (TG) (*n*=3) were 10 years old.

The concentration of total proteins in the CSF and plasma was similar in both groups. The mean value and standard deviation of serum proteins in IVDHG was 66.50±5.20 g/L while in TG was 67.67±2.89 g/L. In both groups, only one subject had sufficient amounts of proteins for accurate measurement. A subject in the IVDHG group had a protein concentration 0.24 g/L, which is within physiological parameters. Two dogs in the TG group had protein concentrations less than instrument detection, as both cases were first stage osteosarcoma in which the tumor had not broken the hematoencephalic barrier. Protein concentration in third dog, which was in the terminal phase, was 52 g/L, which was statistically significantly higher than normal values. Dogs with disk herniation had CFS lactate concentration of 1.49±0.55 mmol/L, while serum concentration was 1.58±1.13 mmol/L. In TG, the mean lactate concentration in CSF was 3.48±1.89 g/L and in serum was 3.98±2.70 g/L. From the results obtained for CSF-lactate and serum-lactate, it is apparent that animals in TG have more than twice the lactate concentrations than normal values. The mean glucose concentration in CSF was 3.46±0.76 mmol/L in IVDHG, while serum concentration was 6.43±0.62 mmol/L. In TG, the mean glucose concentration in the CSF sample was 4.75±3.13 g/L while in serum was 5.76±2.39 g/L. Glucose serum concentration was similar in both groups and was within the range of physiological parameters. CSF glucose concentration was statistically significant higher (approx. 40%) in TG than in IVDHG, which fell within physiological parameters.

Discussion

During CSF sampling, anesthesia was maintained only with propofol owing to its properties of lowering cerebral blood flow and intracranial pressure, and blood pressure (Harvey et al., 2007).

In clinically healthy dogs, the normal protein content is 0.12-0.40 g/L in CSF and is 54-75 g/L in serum, and most proteins are albumins (Brooks and Adams, 1975). Change in concentration is related to a damaged blood-brain barrier (BBB) or a local IgG increased production within the CNS. Elevated protein levels are therefore found in inflammatory/infectious, toxic/metabolic, vascular and neoplastic diseases (Dewey and Ducote, 2008). In this study, plasma protein concentrations in both groups were within physiological parameters. It was not possible to determine accurate concentration of proteins in CSF due to instrumental limitations (>1 g/L). Only two patients, one in each group, had measurable amounts of proteins. These results suggest that disk hernia does not affect CSF protein concentrations.

Patients with stage I osteosarcoma had protein concentrations within normal values, though these values grew after the sarcoma damaged the hematoencephalic barrier. The patient in the terminal phase had a concentration of 52 g/L, which is statistically higher than normal values (130 times more). This suggests that a significant increase in protein content will be observed only in the case of a breakage of the BBB, allowing proteins with a higher molecular mass than albumins and/or protein-inducing tumors to pass the barrier.

The CSF glucose level is dependent upon blood glucose level and the rate of metabolism in the CNS, and therefore for diagnostic purposes, CSF and blood glucose levels should be obtained simultaneously (Kulkarni et al., 2009). CSF levels are usually 40-50% of blood levels. The glucose serum concentration is in the range 4.2-6.6 mmol/L. Plasma glucose concentration in both groups was within physiological parameters. A difference was observed in the glucose concentration obtained from CSF samples. While glucose concentrations in IVDHG was within the normal values, as reported by Witsberger et al. (2012), in TG, a statistically significant increase of approximately 40% in relation to normal values was observed. From the literature, it is known that glucose levels in CSF are dependent on blood glucose levels, selective permeability of the blood to the CSF barrier, and the presence or absence of a glycolytic barrier (Kulkarni et al., 2009). An increased glucose level in the CSF is termed as "hyperglycorrhacia" and is associated with diseases having hyperglycemia, such as spinal cord compression, brain tumors or brain abscess. This study also confirmed that increase of CSF-glucose concentration as a possible tumor marker.

In dogs, lactate production has been estimated at 1.4 to 2.4 mmol/kg BW body weight per hour, so the concentration of plasma lactate is 2.5 mmol/L (Caines et al., 2013). CSF lactate concentration in healthy dogs is 1.55 mmol/L. The principal source of CSF lactate is brain glycolysis, because at normal pH values, lactate is ionized, thereby limiting its transfer across the BBB (Venkatesh et al., 2000). CSF and serum lactate concentration in TG was statistically significant higher 3.48±1.89 g/L (> 100%) respectively 3.98±2.70 g/L (60%) than normal values. A number of factors may contribute to the elevated concentrations of CSF lactic acid, though this has not been observed in patients with infectious disease and disk herniation (Paulson et al., 1971; Zupping et al., 1971; Hansen et al., 1974; Brooks and Adams, 1975). The increase may be due to any of the following: the presence of leukocytes, organisms, tumors or increased production by cerebral tissue secondary to hypoxia. Elevated CSF lactate concentration in patients with a tumor is most likely due to relative ischemia and necrosis. In the IVDHG group, a statistically significant decrease of plasma lactate concentration (1.58±1.13 mmol/L) by approximately 60% compared to normal values was observed, while the CSF concentration (1.49±0.55 mmol/L) was within normal vales, in accordance with the results of Witsberger et al. (2012). This finding is in agreement due to fact that blood and CSF lactate generally vary independently of each other (Gurdjian et al., 1946; Posner and Plum, 1967; Deisenhammer et al., 2011).

Conclusions

The study results showed that a significant increase in protein content will be observed only in the case of a break of the hematoencephalic barrier, and therefore this parameter cannot be used as a specific marker. While serum glucose concentrations in both groups were within physiological

parameters, differences were observed in CSF. Glucose concentrations in TG were significant increased by approximately 40% compared to values in the IVDHG group, which were within the physiological range. This suggests that an increase of CSF-glucose concentrations has possible application as a tumor marker. CSF and serum lactate concentrations in TG were statistically significant higher (3.48±1.89 g/L (> 100%), 3.98±2.70 g/L (60%), respectively) compared to physiological values. On the other hand, in the IVDHG group, a statistically significant decrease of plasma lactate concentration (1.58±1.13 mmol/L) by approximately 60% was observed compared to physiological values. Therefore, it can be concluded that the concentrations of glucose and lactate could be used as a rapid diagnostic tool. An elevated concentration of lactate in serum, while glucose concentration is within the physiological range, indicates tumor. On other hand, a significant decrease of lactate concentration in serum while glucose concentration is within the physiological range indicates disk herniation. In patients with tumor, a significant increase of glucose concentration in CSF will be observed, while in disk herniation, both parameters will be within the physiological range.

References

1. BELDA, B., A. LARA-GARCIA and P. LAFUENTE (2016): Canine appendicular osteosarcoma. Vet. Ireland J. 7, 207-216.
2. BROOKS, B. R. and R. D. ADAMS (1975): Cerebrospinal fluid-acid-base and lactate changes after seizures in unanesthetized man. Neurology 25, 935-942.
3. CAINES, D., M. SINCLAIR, D. WOOD, A. VALVERDE, D. DYSON, L. GAITERO and S. NYKAMP (2013): Evaluation of cerebrospinal fluid lactate and plasma lactate concentrations in anesthetized dogs with and without intracranial disease. Can. J. Vet. Res. 77, 297-302.
4. DEISENHAMMER, F., A. BARTOS, R. EGG, N. E. GILHUS, G. GIOVANNONI, S. RAUER, F. SELLEBJERG and H. TUMANI (2011): Routine cerebrospinal fluid (CSF) analysis. In: European Handbook of Neurological Management, (Gilhus, N. E., M. P. Barnes and M. Brainini, Eds.) Blackwell Publishing Ltd., 2nd edition. Pp. 5-17.
5. DEWEY, C. W. and J. M. DUCOTE (2008): Neurodiagnostics. In: A partical guide to canine and feline neurology, (C. D. Dewey, Ed.), Wiley-Blackwell publishing, A John Wiley and Sons ltd production, 2nd edition. Pp. 75-115.
6. GURDJIAN, E. S., J. E. WEBSTER and W. E. STONE (1946): Cerebral metabolism in metrazol convulsions in the dog. Res. Publ. Assoc. Res. Nerv. Ment. Dis. 26, 184-205.
7. HANSEN, E. L., H. S. KRISTENSEN, P. BRODERSEN, O. B. PAULSEN, S. MÜLLERTZ and O. JESSEN (1974): Acid-base patterns of cerebrospinal fluid and arterial blood in bacterial meningitis and in encephalitis. Acta Med. Scand. 196, 431-437.
8. HARVEY, R. C., S. A. GREENE and W. B. THOMAS (2007): Neurological disease. In: Lumb and Jones' Veterinary anesthesia and analgesia (W. J. Tranquilli, J. C. Thurmon, K. A. Grimm, eds.); Blackwell publishing, 4th edition, pp. 903-913.
9. KHASAWNEH, A. H., R. J. GARLING and C. A. HARRIS (2018): Cerebrospinal fluid circulation: What do we know and how do we know it? Brain Circ. 4, 14-18.
10. KULKARNI, M. D., S. R. SAMANT, G. B. YADAV, A. V. KHANVILKAR and M. W. KHASNIS (2009): Diagnostic Importance of Cerebrospinal Fluid in Pathognomonic Condition. Vet. World 2, 441-443.
11. PAULSON, G. W., G. E. LOCKE and D. YASHON (1971): Cerebral spinal fluid lactic acid following circulatory arrest. Stroke 2, 565-568.
12. POSNER, J. B. and F. PLUM (1967): Independence of blood and cerebrospinal fluid lactate. Arch. Neurol. 16, 492-496.
13. VENKATESH, B., P. SCOTT and M. ZIEGENFUSS (2000): Cerebrospinal Fluid in Critical Illness. Crit. Care Resusc. 2, 42-54.
14. WITSBERGER, T. H., J. M. LEVINE, G. T. FOSGATE, M. R. SLATER, S. C. KERWIN, K. E. RUSSELL and G. J. LEVINE (2012): Associations between cerebrospinal fluid biomarkers and long-term neurologic outcome in dogs with acute intervertebral disk herniation. JAVMA 240, 555-562.
15. ZUPPING, R., A. E. KAASIK and E. RAUDAM (1971): Cerebrospinal fluid metabolic acidosis and brain oxygen supply-studies in patients with brain infarctions. Arch. Neurol. 25, 33-38.

Analiza cerebrospinalne tekućine u pasa s kralježničnim tumorom i hernijacijom intervertebralnog diska

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Ovo istraživanje predstavlja retrospektivnu studiju. Cilj ovog istraživanja bio je ustvrditi mogu li laktat, glukoza ili ukupni protein u serumu i cerebrospinalnoj tekućini biti specifični biokemijski markeri u brzoj diferencijalnoj dijagnostici kralježničnog tumora i hernijacije intervertebralnog diska. U istraživanju je korišteno 9 uzoraka pseće krvi i 9 uzoraka pseće cerebrospinalne tekućine koje su biokemijski analizirane. Ukupno 9 je pasa podijeljeno u 2 skupine: hernijacija intervertebralnog diska (IVDHG) (n=6) i skupina vertebralni tumor (TG) (n=3). Za kvantitativno određivanje koncentracija laktata, glukoze i ukupnih proteina u serumu i cerebrospinalnoj tekućini koristila se metoda apsorpcijske spektrofotometrije. Koncentracije laktatata u serumu i cerebrospnalnoj tekućini i glukoze u cerebrospinalnoj tekućini su bile povišene za 40 do 60 % u pasa s tumorom u kralježnici u usporedbi s psima iz skupine s hernijacijom intervertebralnog diska. Serumska koncentracija laktata u pasa s hernijacijom intervertebralnog diska je bila 60 % niža nego fiziološke vrijednosti. Rezultati ove studije su pokazali da povećane koncentracije glukoze, laktata i ukupnih proteina u cerebrospinalnoj tekućini upućuju na tumor u kralježnici, dok, smanjene ili fiziološke vrijednosti laktata i ostalih mjerenih pokazatelja mjerenih u serumu i cerebrospinalnoj tekućini ukazuju na hernijaciju intervertebralnog diska.

Ključne riječi: *pas, cerebrospinalna tekućina, kralježnični tumor, hernijecija međukralježničnog diska*