High resolution magic angle spinning 1H NMR spectroscopic investigation of listeria brainstem encephalitis in small ruminants: preliminary results

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Purpose

Neurolisteriosis is caused by infection with *Listeria monocytogenes* (LM) and is associated with a high mortality rate in humans and ruminants. Listeria brainstem encephalitis is the most common form in ruminants, and its neuropathology is strikingly similar to that in humans.[1] Although immune reactions to LM have been studied in detail[1], little is known about metabolic changes associated with neurolisteriosis in particular and inflammatory diseases of the central nervous system (CNS) in general[2]. High resolution magic angle spinning (HR-MAS) NMR spectroscopy is an established technique to study small molecule metabolites of tissue biopsies and has given valuable biochemical insight in neoplastic CNS diseases.[2] The purpose of our study was to investigate metabolic changes associated with listeria brainstem encephalitis in small ruminants as a model for an inflammatory CNS disease.

Methods

Bilateral brainstem and thalamus biopsies (4.2-13.9mg) were obtained from 7 healthy control animals (6 sheep, 1 goat) and 8 spontaneously diseased animals (4 sheep, 4 goats) diagnosed with listeria brainstem encephalitis by post mortem histopathological examination. After minimally invasive stereotactic brain biopsy under general anesthesia, all animals were euthanized. The biopsies were immediately snap-frozen in liquid nitrogen and stored at -80°C, before being placed in a 12 µl MAS rotor with D_2O-based phosphate-buffered saline. ^{1}H HR-MAS NMR experiments with water presaturation applying the 1D Carr-Purcell-Meiboom-Gill (CPMG) sequence (*cpmgpr1d*) were performed on a Bruker Avance II spectrometer (500.13 MHz) at 4 kHz MAS and 285 K. After postprocessing of spectra using TopSpin (3.1, Bruker Biospin GmbH), chemometric analysis to determine differences between diseased and control groups was performed using Matlab (R2011b, The MathsWorks Inc.). For multivariate analysis by Principal Component Analysis (PCA) and Partial Least Squares Regression (PLS), 35 buckets of variable size according to peak width were defined after exclusion of areas of pure noise, lipids and the contaminant ethanol (aseptic skin preparation). Sections of the brain at the area of biopsy were investigated histopathologically using Haematoxylin and Eosin staining, inflammatory infiltrates were graded as mild, moderate or severe. Experiments were performed in agreement with the local ethics regulations.

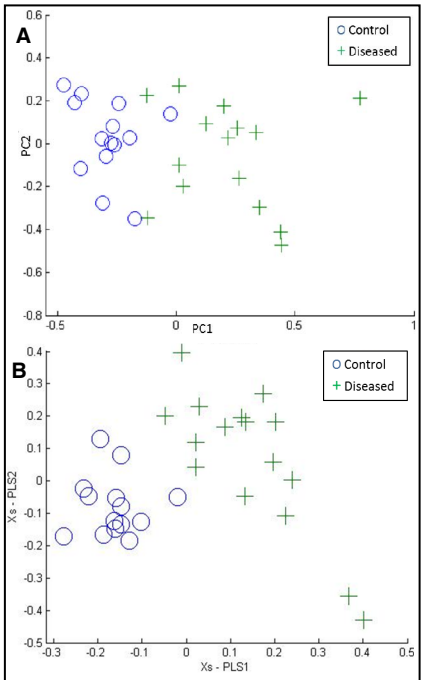

Fig. 1 PCA (A) and PLS (B) scores plot for brainstem biopsies from control and diseased animals

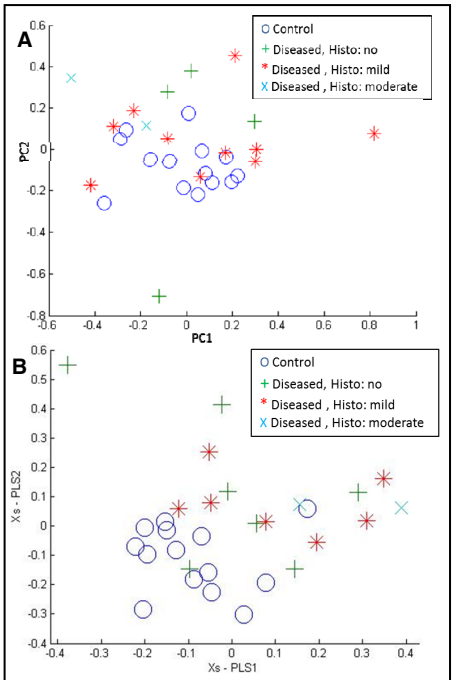

Fig. 2 PCA (A) and PLS (B) scores plot for thalamus biopsies from control animals and diseased animals, grouped by the grade of histopathological changes

Results

Histopathologically, all animals of the diseased group showed moderate to severe inflammatory changes in the brainstem. In contrast, in the thalamus of diseased animals no inflammatory infiltrates were observed in 2 animals bilaterally and in 3 animals unilaterally. Mild changes were detected in 2 animals bilaterally and 3 animals unilaterally, and moderate changes in 1 animal bilaterally. Chemometric analysis of the brainstem biopsies achieved near complete separation of the diseased and control group in unsupervised PCA (Fig.1A), and a complete separation in PLS (Fig.1B) with >95% explained variance by the first two principal components. According to the loading plots, important discriminators were N-acetylaspartate, choline, phosphocholine, glycerophosphocholine and lactate. In contrast, for the thalamus biopsies no separation between the control and diseased group could be achieved by PCA (Fig.2A). However, taking the grade of histopathological changes into account, a trend towards a clustering of the control and diseased group can be observed in PLS (Fig. 2B).

Discussion

Even though only a small data set was available for analysis, differences in the metabolic profile in the primarily affected location, the brainstem, could be identified by NMR spectroscopy and multivariate analysis, and will be used to build a model to be tested on a second data set. Not surprisingly, in the commonly unaffected or mildly affected thalamus no separation between the groups could be achieved without the use of prior knowledge by PCA. However, the trend towards a clustering of the control group and more wide distribution of the diseased group predominantly showing no or mild histopathological changes with the use of prior knowledge by PLS may indicate the high sensitivity of NMR spectroscopy to detect metabolic changes, even before histopathogically observable inflammatory infiltrates occur.

Conclusion

HR-MAS NMR spectroscopy identified differences in the metabolic profile of brainstem biopsies in small ruminants diagnosed with listeria brainstem encephalitis and has the potential to be used as a model to improve the molecular characterization of inflammatory CNS diseases. Further investigation is needed to support these preliminary results and assess the underlying molecular pathways.

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References 1. Oevermann et al., Interdiscip Perspect Infect Dis, 2010. 2. Lindon et al., Prog Nucl Mag Res Sp, 2009. 55(2), 79-100.