Quantitative analysis of metabolic alterations in a mouse model of neuro-inflammation using *in vivo* MR Spectroscopy

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Introduction

Quantification of the NMR spectroscopic observable metabolites can provide considerable biochemical information and can help clinical investigators in understanding the role of metabolites in normal and pathological conditions associated with neuro-inflammation disease. *In vivo* MR metabolic investigations will contribute to determine how regulatory metabolic processes work in order to prevent neuronal injury. The aim of the present study was to monitor the metabolic changes in a mouse model of neuro-inflammation. Magnitude and duration of metabolic alterations and/or recovering on cohorts of infected mice were investigated.

Method

Description of the disease model: the model of neuro-inflammation was obtained and validated by INSERM's team [1, 2]. Sixteen female mice (Swiss, 4 weeks old, 17g in weight) were injected intracerebrally with the Canine Distemper Virus, a virus closely related to the human measles virus. Acute encephalitis develops 7 days following inoculation, peaks 14 days post-inoculation, and is related to the early viral replication that took place electively in neurons located in hippocampus, hypothalamus and monoaminergic nuclei (substantia nigra, raphe dorasalis and locus coeruleus). Animals that survive the acute phase tend to develop late neurological disorders such as paralysis and obesity. The hippocampus of infected (n=11) mice were investigated at D3 and D8 after the inoculation using ^{1}H MRS as well as age-matched sham control mice (n=15). During the MRS experiments, the mice were anesthetized by inhalation of a gas mixture of air and isofluorane. The respiratory cycle was monitored using a pressure probe and the body temperature was maintained at 37°C by with the electronic temperature control of the animal holder. Experiments were conducted according to the procedures approved by the Institutional Animal Care and Ethical Committee of our University.

***Experimental conditions**:* The experiments were performed on a horizontal 4.7T Biospec BRUKER system. Acquisitions were performed using a short–echo time PRESS sequence (TE/TR=20/5000ms, Tacq=21min, 4096 data-points, bandwidth of 4kHz). The localization of the VOI was based on T_2-weighted RARE images (TR/TE=3510/70.6ms, slice thickness 1mm, RARE factor=8, field of view=25.6x25.6mm, matrix=256x192). First- and second- order shim terms were adjusted using FASTMAP for each volume of interest centered in the hippocampus (right side of the brain, 2.5x2x2mm^3). Eddy current compensation and static magnetic field drift correction were applied during the acquisitions. VAPOR [3] interleaved with OVS pulses was used in order to suppress the water and outer volume signals. The unsuppressed water signal was also acquired for each mouse.

Quantifications: The MRS signals were processed in the time-domain. Residual eddy current effects were corrected and the residual water component was eliminated using HLSVD. The metabolite concentrations were estimated using a modified version of the semi-parametric QUEST method [4, 5]. The following fourteen metabolite signals were included in the simulated metabolite basis-set: aspartate (Asp), choline (Cho), creatine/phosphocreatine (Cr), gamma amino butyric acid (GABA), beta glucose (β-Glc), glutamate (Glu), glutamine (Gln), glutathione (GSH), glycine (Gly), lactate (Lac), myo-inositol (mIns), N-acetylaspartate (NAA), scyllo-inositol (sIns) and taurine (Tau). Ten extra Gaussian lineshape signals were included in the basis-set to model the macromolecule and lipid contributions. The metabolite concentration estimates were normalized using the unsuppressed water signal, used as an internal reference. Statistical differences between the shams and the infected mice with regard to the concentration estimates were determined by the non-parametric Mann–Whitney U-test.

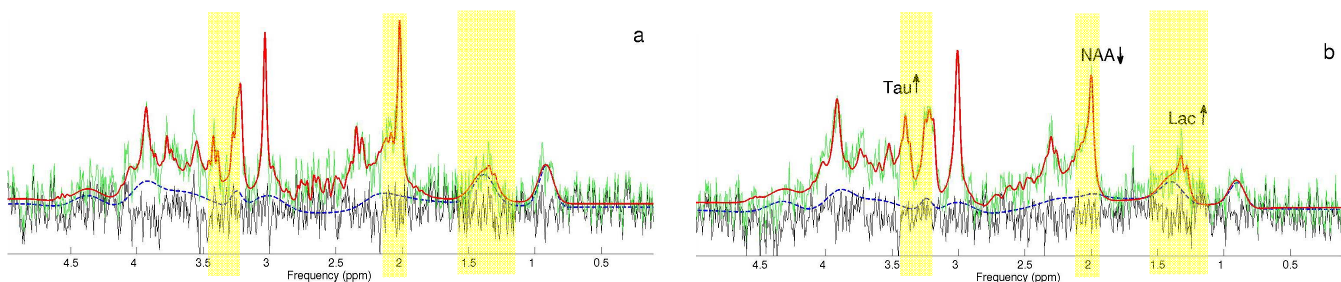

Figure 1: ^{1}H water-suppressed spectra acquired at 4.7T from the hippocampus of a) a sham and b) an infected mouse at D3: raw spectra (green line), estimated spectra (red line), estimated background spectra (blue line) and the residues (black line). Increases of Tau and Lac and decrease of NAA are shown.

Results

In the first two weeks after the virus inoculation, the behavior of the infected mice was like that of the healthy mice. Then the mice lost a quarter of their weight in one week and became less mobile and progressively paralyzed. The mortality rate was 81% and 94% at D21 and D28, respectively. A continuous decrease of the total NAA was detected beginning at D3 (D3, -15%, p<0.05) compared to the concentration quantified for the shams. A statistically significant increase of Tau concentration was quantified at D3 (+20%, p<0.05), which at D8 returned to the concentration observed in shams. At D3 and D8, increases of Lac (D3, +57%, p<0.05; D8, +53%, p<0.05) were detected. Increased amplitude of the group of resonances around 3ppm (GSH+Cr+MM3) was noticeable at D3 and D8 without reaching significance. No significant changes were observed for the concentration of Cho.

Discussions/Conclusions

Significant concentration changes of total NAA - a neural density and integrity marker, Lac - an anaerobic metabolism marker, and Tau - released in considerably higher quantities in hypoxia, were detected from the early stages of the neuro-inflammation. The decrease of NAA has been described in neuronal insults and may sign a neuronal suffering virus-induced. Taurine has been thought to be essential for the development and survival of neural cells and to protect them under cell-damaging conditions and its release could constitute an important mechanism against excitotoxicity. High concentration of Lactate may indicate alterations of oxidative phosphorylation seen in pathological condition of anaerobic metabolism. In conclusion, MRS is useful to detect metabolic dysfunctions from the early stages and investigate the outcome of neuro-inflammation disease.

References

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