Hyperammonemia and edema: a DTI study in the adult rat brain

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Introduction:

Ammonia is a neurotoxin implicated in the pathogenesis of hepatic encephalophaty (HE). HE is a serious neuropsychiatric condition resulting from both acute (ALF) and chronic liver failure. Brain edema, with associated increased intracranial pressure and brain herniation represents the major complication of ALF [1]. It is not yet clear whether brain edema is mostly vasogenic or cytotoxic. Published data on both, humans and animal models of hyperammonemia associated with experimental acute liver failure strongly support that brain edema is mostly cytotoxic due mainly to astrocyte swelling [1,2]. It is generally accepted that hyperammonemia plays a critical role in this process; however the mechanism is still incompletely understood. In this context, the aim of this study was to assess the effects of hyperammonemia alone on the rat brain by using DWI.

Materials and Methods:

Four SD rats (300-350g) were fasted overnight before the experiment. The femoral artery and vein were catheterized for blood sampling, as well as ^{14}N ammonium chloride and α-chloralose infusions. The rats were artificially ventilated. After giving a bolus over 1 min, ^{14}N ammonium chloride was then infused continuously at a stable rate (4.5mmol/h/kg) for up to 6-7h. Body temperature was maintained at 37.5 ^{0}C using thermoregulated water circulation. All MR experiments were performed on a 9.4T/26cm magnet (Varian/Magnex) equipped with 12-cm gradient coils (400mT/m, 120μs) with a quadrature transmit-receive 16-mm surface RF coil. Diffusion tensor acquisition was done with double spin echo semi-adiabatic 4 shots EPI sequence [3] (FOV: 23 × 15 mm^2, Acq matrix 128 × 64, 5 slices, 1 mm thickness, 14 averages with TE/TR = 42/2000 ms). Diffusion gradients (G_{diff} = 21 G/cm, δ = 3 ms and Δ = 20 ms, giving a *b*-value of 1029 s.mm^{-2}) were applied along 6 directions. Total acquisition time was less than one hour. Diffusivity values (ADC, FA) were derived from the tensor using a Matlab (Mathworks, Natick, MA) script. ADC was measured in ROIs positioned in four brain regions on: the cortex, the striatum, the hippocampus and the ventricles. Ventricles sizes were calculated by drawing ROIs on the ADC maps with a threshold set to 10 x10^{-4} mm^2/s. All data are reported as mean ± SD over the four animals. Three DTI acquisitions were performed: A. before starting the ^{14}N ammonium chloride infusion as reference, B. +3h after infusion and C. +6h after infusion.

Results:

Images obtained with semi-adiabatic EPI sequence had high SNR and coverage of the brain and were free of susceptibility artifacts as well as major geometric distortions (c.f. Fig. 1). DTI acquired before the infusion shows apparent diffusion coefficient (ADC) in the cortex (ADC = 8 ± 0.5 ×10^{-4} mm^2/s), striatum (ADC = 7.5 ± 0.6 ×10^{-4} mm^2/s) in excellent agreement with literature [4]. A noticeable increase in the ventricle size is visible in the ADC maps at 3 hours and 6 hours of infusion (c.f. Fig. 2, V_{before} = 18 ± 6 ml, V_{+3h} = 29 ± 12* ml, V_{+6h} = 31 ± 13* ml, *: p< 0.05 paired t-test), however the volume appeared to stabilize at 6 hours, and in a few rat a small regression was noticed. In addition ADC measurements, performed in the Cx, Hip and St, showed a decrease, however the reduction was significant only 6 hours after infusion with on average a decrease of 7% in every regions (p < 0.05, non-parametric Friedman test) was noted.

Discussion and conclusions:

The rapid change in ventricles size in the first 3 hours of infusion suggests the action of an osmotic mechanism, which would increase the cerebral spinal fluid in the ventricles. It is unlikely the result of a diminution of the cerebral tissue, releasing space for the ventricles, as this mechanism results generally from long-term insults [5]. In addition, the slight decrease in ADC at +3h (4%), suggesting a restriction in water self-diffusion, would be in agreement with a compression of the cerebral tissue due to an increase in CSF. Afterwards, the continuous decrease of ADC measured at +6h with a stabilization of the ventricles size suggests the presence of process counteracting the pressure from the ventricles. Hyperammonemia associated with experimental acute liver failures is well known to induce brain edema, due to the astrocyte swelling. Consequently, we propose that hyperammonemia per se induces late in time mild cytotoxic edema [6], as indicated by the decrease in ADC at +6h. Nevertheless, the origin of the increase of the ventricles and the presence of mild brain edema remains unclear and will need further investigation.

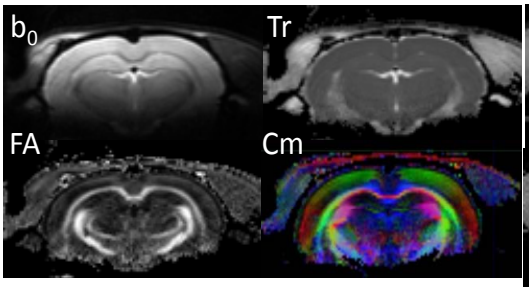

Fig 1. Images acquired with the semi-adiabtic SE-EPI before infusion, with the diffusion coefficient maps. **b_0** image (b = 0 mm/s) , diffusion tensor trace (**Tr**), fractional anisotropy map (**FA**), color encoded map (**Cm**).

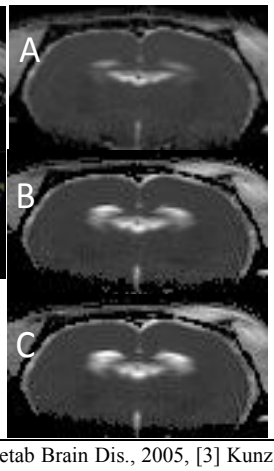

Fig 2. Diffusion tensor trace maps acquired **A.** before infusion, **B.** +3h after infusion, **C.** +6h after infusion. A clear increase of the ventricles size is visible

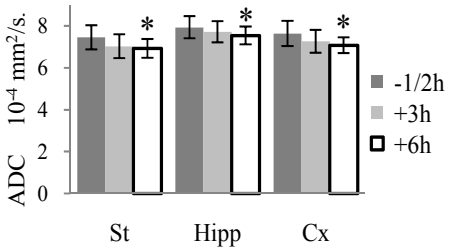

Fig 3. Mean ADC values before infusion, 3h and 6h after infusion in the striatum (**St**), hippocampus (**Hipp**) and cortex (**Cx**). *: p < 0.05 compared to control.

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