Establishing a Method of ^{23}Na - Imaging after Blood Brain Barrier Disruption

Andreas Neubauer[1], Awais Akbar Bajwa[2], Michaela Ruttorf[1], Michael Schwerter[1], Lothar Schilling[2], and Lothar Schad[1]

[1]*Computer Assisted Clinical Medicine, Medical Faculty Mannheim, Heidelberg University, Mannheim, Baden-Wuertemberg, Germany,* [2]*Division of Neurosurgical Research, Medical Faculty Mannheim, Heidelberg University, Mannheim, Baden-Wuertemberg, Germany*

Introduction

Ischemic stroke leading to a shut-down of oxygen and glucose supply impairs cellular function in many ways, and the loss of electrolyte balance is not the least one. With the cessation of the Na/K-ATPase activity Na^+ ions enter and K^+ ions leave the intracellular space eventually resulting in a net increase of the Na^+ and decrease of the K^+ content of brain tissue [1]. Recently, ^{23}Na Magnetic Resonance Imaging (^{23}Na – MRI) has become a useful tool to study these pathological processes *in vivo*. Unfortunately, conventional sequences lack the ability to reliably differentiate between intra- and extracellular sodium. This problem may be overcome by the use of shift agents. Here we present a method to transiently open the blood brain barrier (BBB) and load the brain tissue with the sodium shift agent Thullium (III) (1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetra(methylene phosphonate)) ($Tm[DOTP]^{5-}$). This substance was introduced for *in vivo* ^{23}Na imaging and spectroscopy of the rat brain by Bansal et al. [2]. We used a custom built ^{23}Na surface coil, a Hanning weighted Chemical Shift Imaging (CSI) sequence, capable to acquire the whole Free Induction Decay (FID) in each voxel to follow the alterations of the ^{23}Na signal during the loading period. We calculated T_2* and chemical shift maps which show a strong dependence on the concentration of the shift agent.

Materials and Methods

Animal Preparation: Male Sprague Dawley rats anesthetized with thiobutabarbital (Inactin, 15 mg / 100 g body weight ip) were equipped with a tracheal cannula (for controlled ventilation), and catheters in the right femoral artery (for continuous blood pressure monitoring and withdrawal of blood samples) and in the left carotid artery for bolus injection of mannitol (25% solution, 1.5 ml injected in 45 sec) followed by a slow infusion of $Tm[DOTP]^{5-}$ (80 mM solution, 6 ml over 60 min). At the end of the observation period the blood was flushed from the cerebrovascular system and the concentration of Tm measured in cortical and subcortical tissue samples by means of total reflection X-ray fluorescence analysis (T-XRF) methodology.

Experiments: MRI experiments were performed by use of a CSI sequence with FID readout on a 9.4 T small animal scanner (Biospec®, Bruker, Germany). Parameters of the CSI sequence: T_R = 50 ms, acquisition delay T_{AC} = 0.38 ms, number of scans per experiment = 14340, sampling time for each FID = 400 ms, FoV = 42x71x40 mm³, matrix size = 25x43x25, number of averaged images = 8, time for each scan = 10 min, spatial resolution = 1.7x1.7x1.6 mm³, number of scans = 14. To coregistrate the images two reference tubes with sodium solute were placed at the upper side of the surface coil.

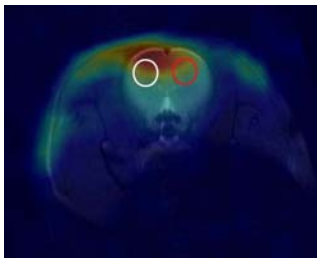

Figure 1: Superimposed ^{1}H/^{23}Na image with ROIs for the left (white circle) and right (red circle) hemisphere.

Post processing: Coregistration of the ^{1}H/^{23}Na data was performed in MATLAB®. In order to compute T_2* maps a mono-exponential least square fit was applied to the FID of each voxel. A Fourier transform was applied to the FIDs and the resonance frequency was determined by identifying the maximal value of the resonance curve under control conditions. Chemical shift maps were created by comparing the resonance frequencies before and during and after infusion of $Tm[DOTP]^{5-}$. Two regions of interest (ROIs) were manually defined in the hemispheres with the help of the anatomical pictures (Fig. 1) and evaluated. The obtained spectra of the ROIs are plotted as exemplified in Fig. 2 d), e), k) and m).

Results and Discussion

With increasing $Tm[DOTP]^{5-}$ loading time the T_2* maps showed a significant decrease of T_2* from 5 – 7 ms to 2 – 4 ms (Fig. 2 b) and h)) which was accompanied by a reduction of the signal intensity (Fig. 2 a) and f)). This reduction in T_2* and intensity may arise from B_0-field inhomogeneities caused by the appearance of the paramagnetic shift agent and thus leading to a rapid dephasing of the spin packages. In parallel with the decrease of T_2* an increase of the chemical shift from around 0 – 0.23 ppm to 0.70 – 1.50 ppm was observed in the left (infused) hemisphere (right hemisphere, appr. 0.46 ppm). The comparison of the spectra obtained in the ROIs in the left (white circles in Fig. 2) and right (red circles in Fig. 2) hemisphere clearly shows a large amount of the extracellular sodium shifted to 1.5 ppm in the left hemisphere (compare Fig. 2 d) and k)) with the signal arising from the intracellular sodium remaining at 0 ppm. In the right hemisphere the shift of the signal is constant about 0 - 0.23 ppm without any obvious separation between intra- and extracellular sodium. In view of our serial measurements in phantoms a shift of 1.5 ppm may be generated by appr. 2 mM $Tm[DOTP]^{5-}$. In fact, based on the T-XRF analyses we could calculate a concentration of 2.30±1.80 mM Tm (mean±SD) in the extracellular space.

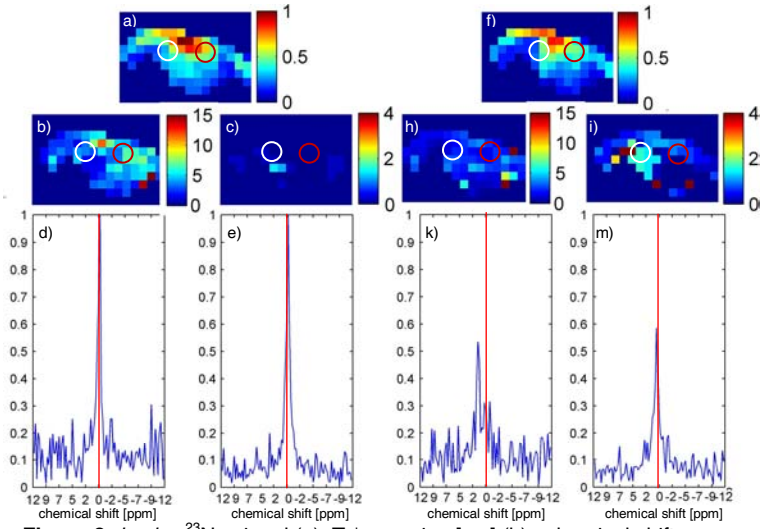

Figure 2: *in vivo* ^{23}Na signal (a), T_2*-mapping [ms] (b), chemical shift mapping [ppm] (c), spectrum in ROI 1 (white circle) (d), and spectrum in ROI 2 (red circle) (e), each before $Tm[DOTP]^{5-}$ infusion; f) – m) show the information depicted in a) – e) after 40 min of infusion

Conclusion

The time course of the chemical shift in the left hemisphere and the decrease in T_2* suggest successful loading of the brain tissue with $Tm[DOTP]^{5-}$ after hyperosmotic opening the BBB. The concentration reached allows the extracellular component of the total Na^+ signal to be distinguished from the intracellular in the brain *in vivo*. In comparison with the values obtained after venous infusion (c.f. [3]), it turns out that we achieved a markedly higher chemical shift on a much smaller time scale.

References

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