In Vivo High resolution Magnetic Resonance Spectroscopy of the Adult Zebrafish Brain at 9.4T

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Introduction

Zebrafish (*Danio rerio*) is increasingly used as model organism for understanding brain diseases including neurodegenerative disorders (1) especially due to similar organization of brain components as that of human. However, investigating the brain metabolites of adult zebrafish *in vivo* has not yet been possible. Precise *in vivo* biochemical information from distinct regions of the zebrafish brain non-invasively can be invaluable for monitoring of disease progression and treatment as well as phenotyping of large number of available zebrafish models of various brain diseases. Magnetic resonance spectroscopy (MRS) enables non-invasive *in vivo* quantification of metabolite concentrations in the brain of model organism (2). However, due to sensitivity issues and its small size localized MRS has not yet been implemented in the zebrafish brain. In this study we have implemented and optimized high resolution localized ^{1}H MRS at 9.4T in conjunction with a strong gradient system, an efficient shimming and the water suppression scheme and obtained for the first time *in vivo* localized MR spectra from zebrafish brain. Furthermore, a ^{1}H spectrum of the zebrafish brain extracts was measured to provide correct chemical shift assignments of various metabolites in the zebrafish brain.

Methods

For *in vivo* MRS measurements, fish was anesthetised by adding 0.001% MS222 (ethyl meta aminobenzoate metanesulfonic acid salt) to pH controlled water. Subsequently fish was transferred to a closed flow-through chamber, which was specially designed to support living zebrafish inside the magnet (3). *In vivo* MR imaging and spectroscopy were acquired using a 9.4-T vertical wide-bore imaging systems equipped with a Bruker Avance console and 1000-mT/m gradients. To select a volume of interest (the voxel positioned in the brain), RARE images were acquired (TR=1500ms, TE= 15ms). MRS voxel (1.5 mm × 1.5 mm × 1.5 mm) were localized in the zebrafiish brain covering most of the brain region. The local field homogeneity was optimized by adjustment of first and second order shim coil current using the FASTMAP sequence which resulted in a line width of 25 – 30 Hz. For localized ^{1}H NMR spectroscopy, the PRESS (Point Resolved Spectroscopy) sequence was optimized. The PRESS sequence was preceded by a VAPOR (Variable Pulse Power and Optimized Relaxation Delays) sequence for global water suppression.

For *in vitro* ^{1}H spectra, the metabolites were extracted from 10 adult zebrafish brains (4). The proton spectra were recorded at 25 ^{0}C with a Bruker 400 MHz DMX NMR spectrometer equipped with a pulsed field gradient accessory (Bruker, Germany) and a 5 mm inverse triple high resolution probe with an actively shielded two gradient coils.

Results and Discussion

The challenges of understanding the detailed mechanisms underlying various brain disorders *in vivo* can be overcome by use of available zebrafish models of neurological disorders (2) in combination with non invasive localized MRS methods that can provide biochemical information from distinct regions of the zebrafish brain. In this study, we applied and optimized ^{1}H MRS sequence at high magnetic field of 9.4T to get localized accesss to the zebrafish brain *in vivo*. A high resolution spectra was obtained from a voxel as small as 3.3 µl placed in the middle of the zebrafish brain (Fig. 1). Excellent separation of resonances from various metabolites including N-acetyl aspartate (NAA), Glutamate (Glu), Glutamine (Gln), Taurine (Tau), Creatine (Cr), Myo-inositol (Ins) and Phosphocholine (PChl) was achieved. A complete metabolite profile and a correct resonance assignment of metabolite in zebrafish brain was achieved using proton NMR (Fig. 2). This study suggests that zebrafish brain has very similar metabolite profile as that of human brain which proves zebrafish as an excellent model organism for human brain disorders.

Conclusion

This study represents the first application of *in vivo* MRS in conjunction with high magnetic field to get localized access to the zebrafish brain *in vivo*. The use of *in vivo* localized MRS in zebrafish brain will be imperative for monitoring biochemical changes during disease progression and treatment using variety of available zebrafish models in the future.

References: (1) J.Joore, A.Timmermans, et al. *Biochemistry and Cell Biology* 75, 601-612 (1997); (2) J.F.A. Jansen, W.H. Backes et al. *Radiology* 240:318-332 (2006); (3) S. Kabli, A. Alia et al, *Zebrafish* 3, 431-439 (2006); (4) J.H.Choi, R.Verpoorte, et al. *Plant Cell, Tissue and Organ Culture* 83, 59-66 (2005).

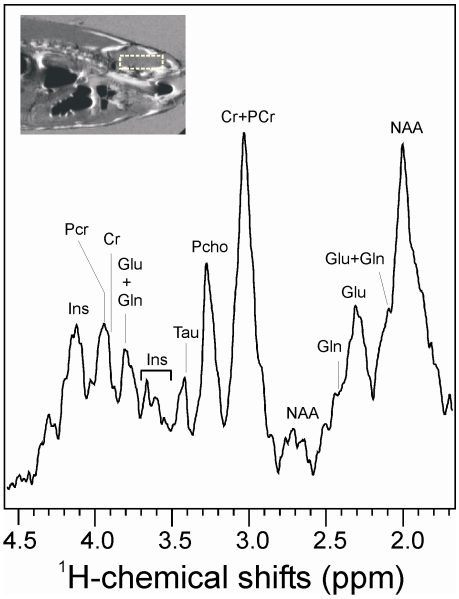

Figure 1: *In vivo* proton local spectroscopy of the metabolites in the brain of the adult zebrafish. 1D ^{1}H MR spectra was obtained from a 3.3 µl voxel placed in the middle of the zebrafish brain as shown in the inset.

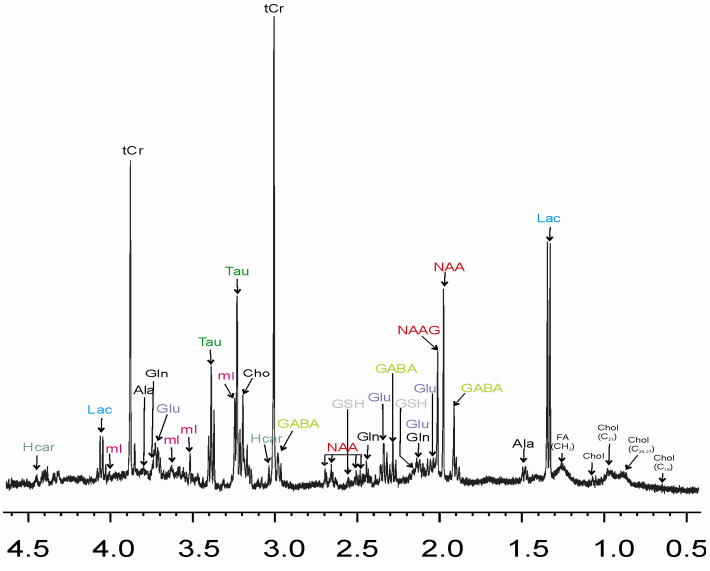

***Figure 2*:** 1D ^{1}H spectrum from the brain extracts of zebrafish.