Qualification of in vivo 1H MRS as a quantitative preclinical tool for evaluating drug efficacy in pharma research

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Introduction: To date, in vivo 1H MRS has been incorporated in numerous preclinical and clinical studies as an alleged biomarker for disease state and treatment efficacy. In the domain of psychiatric disorders, however, the studies have been very heterogeneous in their setups and outcomes have been correspondingly incongruous, particularly in the very few drug studies currently available. In essence, a comprehensive qualification of 1H MRI for its use as a research tool in psychiatric disorders is missing. The goal of the present study was to bridge this gap in preclinical MRS with a tripartite approach: (i) We determined the generic sensitivity of 1H MRS under our standard conditions at 9.4T. (ii) Pharmacological relevance was assessed by establishing dose-response relationships upon pharmacological interventions. (iii) Neurobiological specificity was demonstrated in two functionally distinct brain regions implicated in psychiatric disorders.

Methods: MRS was carried out on a Bruker BioSpec 9.4T/20 cm MR scanner equipped with a 72 mm bird-cage resonator for excitation and a circularly polarized brain surface coil for reception. 1H MR spectra were acquired using PRESS single voxel spectroscopy (TR 2 s, TE 10 ms, spectral width 4 kHz, VAPOR water suppression interleaved with outer volume suppression, 2048 complex points, 512 averages, acquisition time 17 min). Two 16uL voxels were assessed in the right CPu and medial PFC, respectively. Metabolite quantification was carried out using LCModel. For MRS, male Sprague-Dawley rats were maintained under isoflurane anesthesia with continuous monitoring and regulation of body temperature and breathing rate. Pharmacological modulation of neurotransmitter metabolism was achieved with vigabatrin (irreversible GABA-T inhibitior), 3-mercaptopropionic acid (GAD inhibitor), and tiagabine (GAT-1 inhibitor), respectively. Each intervention was tested at three different doses (+vehicle) with 8 animals per dose group.

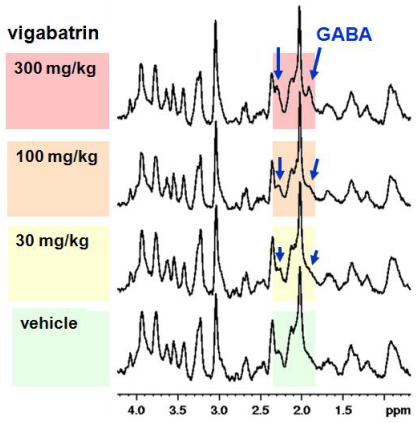

Fig. 1: 1H PRESS spectra from CPu of four animals treated with different doses of vigabatrin.

Results and Discussion: Consistent, highly reproducible spectra were routinely obtained at an average SNR of 12.5±2 and 16.3±2.3 for glutamate in CPu and PFC, respectively (Fig. 1). A prospective power analysis on baseline data projected that minimal changes of 4% in glutamate and 14% in GABA can be detected with a power of 80%, at an alpha level of 5% and a group size of n=8 animals (unpaired t-test), demonstrating that routine preclinical MRS yields discriminative sensitivity in a biologically relevant range.

Drug-induced modulation of the GABAergic system was used to establish the pharmacological relevance of 1H MRS with respect to the sensitivity estimates of our prospective power analysis. Vigabatrin, 3-mercaptopropionic acid and tiagabine are three inhibitors acting on different nodes of the GABAergic system and are expected to elicit large to moderate to small effects on total GABA levels and possible on other neurotransmitters. Concordantly, our results show significant and dose-dependent changes in the directly targeted GABA pool as well as indirectly in glutamate (Fig. 2). The former findings are consistent with biochemistry and microdialysis data in literature and were corroborated by our own ex vivo quantifications with LCMS and drug exposure data obtained from brain and plasma samples. These data testify that preclinical ^{1}H MRS readily detects pharmacologically relevant changes in neurotransmitter pools elicited by direct and indirect modulation.

Moreover, 1H MRS metabolite levels as well as changes induced by pharmacological intervention were found to be highly brain region-specific. With the CPu consisting mostly of GABAergic neurons and the PFC receiving mostly glutamatergic input, these two regions represent functionally distinct entities that 1H MRS can resolve based on their differential neurochemistry.

Conclusion: Our data demonstrate that with appropriate conditions, routine preclinical 1H MRS provides a reliable, quantitative readout of cerebral GABA and glutamate with a discriminating sensitivity well within the biologically and pharmacologically relevant range.

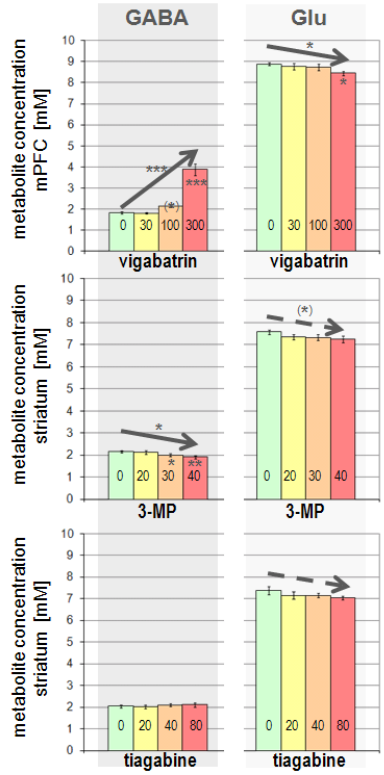

Fig. 2: Dose-response in GABA and glutamate levels upon pharmacological modulation.