Neurotoxicity biomarker development using T_2 mapping in kainic acid excitotoxicity rat model

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Purpose

Current approaches in the analysis of neuropathology in support of new drug submissions to the FDA (the use of 3 or 7 arbitrary histology slices) can sometimes result in false-negative findings, meaning that small localized lesions can easily be missed. Modern non-invasive magnetic resonance imaging (MRI) techniques can provide unique information about the structure and function of the whole brain in vivo with high resolution and contrast in three-dimensional space. The aim of this study was to develop sensitive MRI methods for detecting early neurotoxic changes in the brain of living rats using known neurotoxicant.

Methods

Fifteen male Sprague-Dawley rats (64-75 days old, 369 ± 11 g) were injected with kainic acid (KA, 10 mg/kg, ip) after baseline MRI scans. Animals were continually scanned (T_2 and MRS) for 2 hours and again two days after drug administration. MRI was performed using a Bruker 7 tesla instrument with Bruker 72 mm volume transmit and rat brain surface receive coils. Animals were anesthetized with isoflurane (1-2% @ 1 L/min in oxygen) and the body temperature was maintained at 37.3 ± 0.6°C. For T_2 relaxation mapping of the whole brain, a multi-echo spin echo sequence was used (MTX 192 × 192, number of slices = 24, FOV = 3.84 × 3.84 cm, slice thickness = 1 mm, echo spacing = 15 ms, 16 echoes, TR = 6 s, NA = 1). Proton MRS was acquired in the left dorsal hippocampus (voxel size 4 × 4 × 2 mm) using PRESS sequence (TE = 8 ms, TR = 2.5 s, NA = 128, VAPOR water suppression, with OVS). Resultant T_2 maps were co-registered using a 12-parameter affine transformation (FSL) [1]. MR spectra were processed using LCModel [2]. Baseline T_2 maps were used to obtain combined inter-subject average and standard deviation maps. Each T_2 map obtained after KA administration was compared to the averaged map voxel-by-voxel using Student's t-test to obtain the maps of significant T_2 changes after the treatment and the areas of significant changes were calculated using LabVIEW routines. Histological assessment (80 coronal slices evenly throughout the brain) was performed at baseline, 2 and 48 hours after KA treatment using Amino CuAg stain utilizing relative neurodegeneration ranking (0 - no degeneration, 1 – minimal, 2 – mild, 3 – moderate, 4 – marked degeneration) [3]. Statistical analysis was performed using repeated measures ANOVA and Spearman rank order correlation analysis.

Results and Discussion

T_2 maps were highly reproducible between subjects resulting in very low values of standard deviation (Fig. 1). KA led to significant increases in T_2 values in the hippocampus as early as 2 hours after administration (Table 1). These changes were more pronounced and spread to larger areas including the amygdalae, thalamus, and cortex 2 days after the treatment. Neurodegeneration was revealed in histological examination 48 but not 2 hours after the treatment (Table 1). There was a positive correlation between the area of significant T_2 changes and histopathological scores in KA treated brains (R = 0.853, P = 0.0004). MRS showed slight increase in total amount of glutamate + glutamine immediately after KA injection and lactate accumulation 15 minutes after KA injection (Fig. 2). Glutamate + glutamine and NAA were significantly decreased 48 hours after the treatment, and lactate was still detectable at that time point. KA is a known neurotoxicant with excitotoxic mechanism of action and specifically activate kainate glutamate receptors, which are abundant in the hippocampus and cortex [4]. The rat hippocampal CA3 region is the most vulnerable to excitotoxicity, which was seen here as early and late T_2 changes. T_2 water relaxation is very stable in normal tissue but may change due to edema, cellular damage (necrosis, apoptosis), hemorrhage or other pathologies, when the properties of tissue water change. Quantitative T_2 mapping provides very early and sensitive non-invasive readouts of acute neurotoxicity and has the potential to serve as a basis for future development and qualification of neurotoxicity biomarkers. Such non-invasive biomarkers for the early preclinical detection of neurotoxicity would benefit public health by extending the number of tools available for the safety evaluation of new drugs.

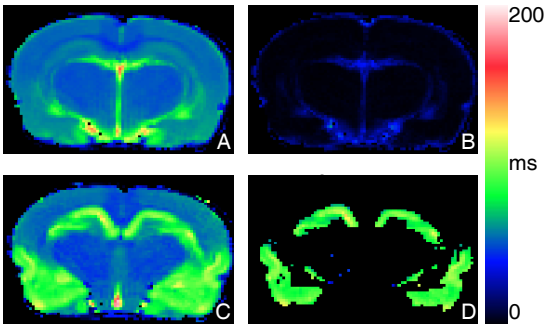

Figure 1. T_2-averaged map of the baseline scans (A) with corresponding standard deviation map (B) and a representative T_2 map of the subject 48 hours after KA treatment (C). The resultant statistical difference map (D) shows significant changes of T_2s in dorsal hippocampus and amygdalae.

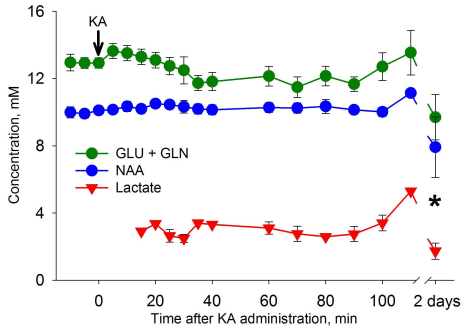

Figure 2. MR spectroscopy showed slight increase of glutamate + glutamine after KA injection (arrow) and lactate appeared in the spectra 15 min after KA. 2 days after KA all NAA, lactate and glutamate + glutamine were significantly lower than at 2 hrs time point (* - P < 0.05).

Table 1. Time course of the changes in T_2 and histological score after KA administration.

Time, hrs	Area of T_2 change, mm^3	Histological score
0	0 ± 0	0 ± 0
2	10.8 ± 2.6*	0 ± 0
48	86.2 ± 34.2*,#	1.84 ± 0.49*,#

* - significant difference compared to 0 hrs
# - significant difference compared to 2 hrs
P < 0.05.

References

1. Jenkinson M. and Smith SM. *Medical Image Analysis*, 5(2), 143-156, 2001
2. Provencher, S. W. *NMR Biomed*, 14(4), 260-264, 2001
3. Switzer RC IIIrd. *Toxicol Pathol*, 28(1), 70-83, 2000
4. Gramsbergen J.B. and Berg, K.J. *Brain Res*. 667: 216-28, 1994