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Full Length Article

Comparison of quantitative T_2 and ADC mapping in the assessment of 3-nitropropionic acid-induced neurotoxicity in rats

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ABSTRACT

To assess the relative performance of MRI T_2 relaxation and ADC mapping as potential biomarkers of neurotoxicity, a model of 3-nitropropionic acid (NP)-induced neurodegeneration in rats was employed. Male Sprague-Dawley rats received NP (N = 20, 16–20 mg/kg, ip or sc) or saline (N = 6, 2 ml/kg, ip) daily for 3 days. MRI was performed using a 7 T system employing quantitative T_2 and ADC mapping based on spin echo pulse sequence. All maps were skull stripped and co-registered and the changes were quantified using baseline subtraction and anatomical segmentation. Following the in vivo portion of the study, rat brains were histologically examined. Four NP-treated rats were considered responders based on their MRI and histology data. T_2 values always increased in the presence of toxicity, while ADC changes were bidirectional, decreasing in some lesion areas and increasing in others. In contrast to T_2 in some cases, ADC did not change. The effect sizes of T_2 and ADC signals suggestive of neurotoxicity were 2.64 and 1.66, respectively, and the variability of averaged T_2 values among anatomical regions was consistently lower than that for ADC. The histopathology data confirmed the presence of neurotoxicity, however, a more detailed assessment of the correlation of MRI with histology is needed. T_2 mapping provides more sensitive and specific information than ADC about changes in the rat brain thought to be associated with neurotoxicity due to a higher signal-to-noise ratio, better resolution, and unidirectional changes, and presents a better opportunity for biomarker development.

1. Introduction

The safety of newly developed drug candidates is the most important reason for the high rates of attrition during preclinical development, which contributes greatly to the increased cost and long time required to bring new medications to the market (Cook et al., 2014). Novel approaches and tools to assess toxicity with high sensitivity and specificity are needed to advance preclinical testing of new drugs (Woodcock and Woosley, 2008). Non-invasive MRI is currently gaining momentum to become such a tool (Pogge and Slikker et al., 2004; Hanig et al., 2014; Liachenko et al., 2015; Liachenko and Ramu, 2017; Ramu et al., 2016). A variety of MRI methods have been claimed to be useful in the assessment of nonclinical neurotoxicity, the most popular being diffusion- and T_2-relaxation-sensitive types (Qiao et al., 2000; Preece et al., 2004; Cauli et al., 2007; Sierra et al., 2011; Dror et al., 2014). However, the question of interest is which method, diffusion or T_2 mapping, is better suited for assessing neurotoxicity with the greatest confidence and efficiency. One of the ways to answer this question is to compare the performance and statistical effect sizes (Cohen, 1988), variability and specificity of toxicity-related signals obtained using different MRI methods on the same subjects exhibiting frank neurotoxicity. That approach was implemented in the present study using 3-nitropropionic acid (NP) as a model neurotoxicant in rats (Lee et al., 2000; Brownell et al., 2004; Lee and Chang, 2004; Roberts, 2005; Akashiba et al., 2008; Bracko et al., 2014; Hanig et al., 2014).

NP is found naturally in many plants, especially legumes and fungi where it most likely serves the purpose of defense against herbivores and possibly as a means of nitrogen fixation (Francis et al., 2013). The toxicity of NP is caused by its irreversible inhibition of mitochondrial succinate dehydrogenase of the Krebs cycle and the electron transport chain (Alston et al., 1977; Coles et al., 1979; Francis et al., 2013). The main target tissue of NP toxicity is the striatum in the brain and the expression of NP poisoning closely resembles Huntington's disease, for which reason this compound is widely used to model this disease in

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animals (Andreassen et al., 2000; Tantucci et al., 2009; Kumar et al., 2012; Sheline et al., 2013).

2. Materials and methods

2.1. Animals

All animal experiments were approved by and performed in accordance with the policy of the National Center for Toxicological Research IACUC. Twenty-six male Sprague-Dawley rats (338 ± 37 g) were used. Animals were administered NP (3-nitropropionic acid, Sigma-Aldrich) or saline (control) for three consecutive days using doses and routes described in Table 1. For MRI animals were anesthetized using isoflurane (3% induction, 1.2–1.8% maintenance in oxygen at 1 L/min) and placed on a water-heated bed inside the scanner. Respiration, peripheral pulse oximetry, and rectal temperature were monitored and maintained within physiological ranges during scans. Imaging was performed immediately before the first dose of NP or vehicle (baseline image), followed by repetition of the imaging procedure 2 and 3 (and up to 7 in some cases) days later.

2.2. MRI

The imaging was conducted using a Bruker BioSpec 7T/30 system equipped with a 12 cm ID gradient insert (440 mT/m, Bruker BioSpin) and a cross-coil RF setup – 72 mm ID transmit and rat brain 4-channel array receive RF coils (Bruker Biospin). For generation of T_2 maps, a multi-sliced multi-echo spin echo sequence was used with the following parameters: matrix 192 × 192, field of view 3.84 × 3.84 cm, 24 slices (no gaps) of 1 mm thickness, 16 echoes with 15 ms spacing, repetition time = 6 s, number of averages = 1. Quantitative T_2 maps were reconstructed using a voxel-by-voxel simple exponential fit (Hanig et al., 2014; Liachenko et al., 2015). For the generation of apparent diffusion coefficient (ADC) maps, a Steszkaj-Tanner diffusion-weighted spin echo pulse sequence was used with the same field of view as the T_2 maps and the following parameters: matrix 128 × 128 (zero-filled to 192 × 192 before Fourier transform to produce the same geometry as T_2 images), 24 slices (no gaps) of 1 mm thickness, echo time = 26.5 ms, repetition time = 6 s, number of averages = 1, two diffusion-weighted images with b-values $b_0 = 2$ s/mm^2 and $b_1 = 1024$ s/mm^2. Quantitative ADC maps were reconstructed voxel-by-voxel using the equation

$$ADC = \ln[I_2/I_1]/(b_1 - b_2)$$

(Minematsu et al., 1992)

where I_1 and I_2 are intensities in the corresponding voxels of images acquired with b_1 and b_2, respectively.

2.3. Image analysis

The resultant T_2 maps were skull-stripped using semi-automatic routines developed in-house and co-registered to the same reference rat brain using a surface registration algorithm from Analyze 12.0 software as described earlier (Liachenko and Ramu, 2017). A randomly selected T_2 map of high quality was selected as a reference image. All diffusion-weighted images (DWI) were acquired during the same imaging session as the T_2 images with a minimum inter-imaging time interval, producing both T_2 and ADC maps in register. Thus, ADC maps were skull-stripped and co-registered to the reference image using skull-stripping and transform information from their T_2 counterparts. All co-registered baseline T_2 and ADC maps were averaged to produce the corresponding maps of coefficients of variation (CV).

To quantify the volumes of the 'lesions' caused by NP, the co-registered baseline maps (T_2 or ADC) were subtracted from the T_2 or ADC maps obtained from the same animals after treatment. To decrease noise, all maps were down sampled to 0.4 × 0.4 × 1.0 mm resolution and only voxels with baseline CV values of <5% for T_2 and <15% for ADC were considered. Voxels exhibiting more than 10% (T_2) or 15% (ADC) change from the baseline were classified as 'lesioned' and used to calculate the 'lesion' volumes. Negative and positive changes from the baseline were analyzed separately.

Reference T_2 maps were manually segmented according to a rat brain atlas (Swanson, 2004) into the major areas: amygdala, caudoputamen, cerebellum, cortex, hippocampus, hypothalamus, insular cortex, globus pallidus, nucleus accumbens, piriform cortex, thalamus, and ventricles. This reference map was filtered to exclude voxels characterized by high variability at baseline (CV > 25%) to account for imperfect co-registration at the tissue boundaries (brain-skull, and brain-CSF) (Liachenko and Ramu, 2017) and it was used as a mask for segmentation of all co-registered T_2 and ADC maps.

2.4. Histopathology

At the end of the last MRI scan, animals were deeply anesthetized (5% isoflurane in oxygen) and perfused transcardially with 60 ml of perfusion wash solution (0.8% NaCl, 0.4% dextrose, 0.8% sucrose, 0.023% $CaCl_2$, 0.034% sodium cacodylate) followed by 200 ml of cacodylate buffered paraformaldehyde solution (4% paraformaldehyde, 4% sucrose, 1.4% sodium cacodylate) as described on the Neuroscience Associates website (neuroscienceassociates.com) (Switzer, 2000). Preparation and staining of histological slides and evaluation of neuronal degeneration in 40 μ brain sections (about 80 evenly spaced sections per brain) was performed by Neuroscience Associates (Knoxville, TN). The tissue was stained using a modified de Olmos cupric silver stain (DeOlmos and Ingram, 1971; Switzer, 2000).

2.5. Statistics

Statistical analyses were performed using SigmaPlot software. Independent *t*-tests were used to compare the volumes of the lesions produced by NP, and a repeated measures ANOVA was used to compare the changes in T_2 or ADC in segmented brain regions. Effect size was calculated using Cohen's d statistics (Cohen, 1988). All MRI data are presented as Means ± Standard Deviation.

Table 1
Experimental groups.

	Treatment	Dose	Route	Age, days	Weight, g	N	Survived	Survived Responders
1	saline	2 ml/kg	ip	66 ± 2	341 ± 41	6	6	–
2	NP	20 mg/kg	sc	65 ± 0	319 ± 34	7	7	0
3	NP	16 mg/kg	ip	65 ± 0	325 ± 20	8	6	3
4	NP	20 mg/kg	ip	71 ± 0	385 ± 14	5	1	1

Data are means ± SDs, ip – intraperitoneal, sc – subcutaneous.