Assessment of Experimental Stroke Lesion Size Using 1T Benchtop MRI

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TARGET AUDIENCE- This research is of particular interest to groups researching stroke therapy, preclinical stroke and those using *in vivo* MRI for the investigation of stroke.

PURPOSE- To investigate the capability of a newly developed 1T "benchtop" MRI system for the *in vivo* assessment of stroke lesion size in rats compared to gold-standard histology measurements.

INTRODUCTION- High-field MRI (≥ 4.7T) is now routinely used to serially assess stroke lesion size *in vivo* in rodents using T2-weighted (T2w) and diffusion weighted imaging (DWI) acquisitions at 24 hours post ischemia [1]. However, high field scanners are expensive to purchase and maintain, and require extensive support from MR physicists [2]. Recently developed low-field benchtop preclinical MRI systems are cryogen free, can be sited in normal lab situations with a small footprint and do not require extensive infrastructure such as a Faraday cage or liquid helium cooling. Hence, low-cost benchtop alternatives are potentially more cost-effective and could increase the accessibility of MRI to the preclinical stroke community. Therefore, the aim of this study was to determine the feasibility of using benchtop MRI in the assessment of ischemic stroke lesion volume in rats.

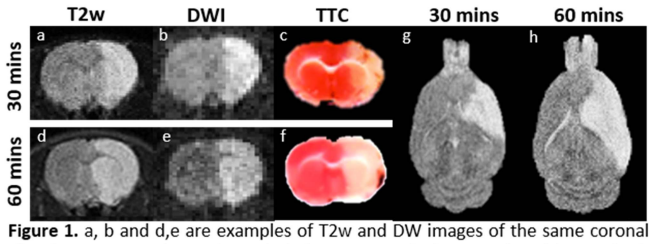

Figure 1. a, b and d,e are examples of T2w and DW images of the same coronal slice from a 30 and 60 min occluded rat, respectively. Hyperintesities on both T2w and DW images correspond to areas of vasogenic and cytotoxic oedema, respectively and are biomarkers of ischemic stroke at 24 hours. c and f are scanned TTC slices of corresponding MRI coronal slices of 30 and 60 mins. White regions represent ischemic tissue and red regions, healthy tissue. g and h are representative T2w transverse slice images of both 30 and 60 min rats with hyperintense pathology observed in the MCA supplied region of the brain.

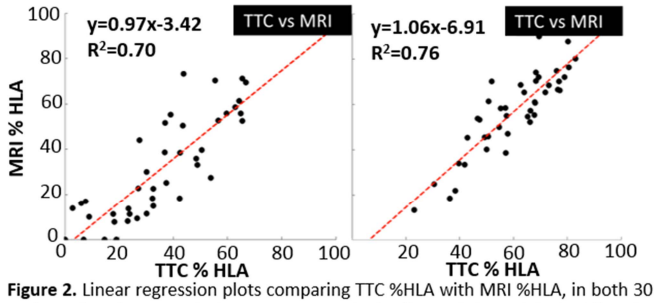

Figure 2. Linear regression plots comparing TTC %HLA with MRI %HLA, in both 30 and 60 min cohorts. Each dot represents a single slice and the red dashed line the regression equation. Correlations for both cohorts were significant ($p<0.05$).

METHODS- Adult male Sprague-Dawley rats (n=12) underwent middle cerebral artery (MCA) occlusion surgery using an adapted intraluminal filament method [3]. Focal ischemia was induced for 30 (n=6) or 60 mins (n=6) followed by reperfusion. 24 hrs following surgery MRI was conducted using a 1T Bruker Icon Scanner (permanent magnet, gradient strength 450mT/m) with a 44mm rat body RF coil. Temperature and respiration were maintained at physiological levels.

T2 Weighted imaging- 15 x 1.25mm coronal slices spanning the whole brain were acquired with a T2 RARE (factor = 8) sequence, FOV=3.5x3.5mm, NEX=9, TR/ETE=2568/60ms(inter-echo spacing=10ms), DM=128[2], scan duration=6m9s.

Diffusion Weighted imaging- 7 x 1.25mm coronal slices, DTI-EPI 8 segment sequence, FOV=3.5x3.73mm, NEX=6, TR/TE=3500/36ms, b-values=0, 800s/mm[2] in Gx, Gy and Gz directions, DM=96x64, scan duration = 12 m.

MRI Analysis- MRI data was analysed using MATLAB. We adapted a novel method for evaluating stroke lesion size that accounts for cerebral swelling, and requires T2w and DW data [4]. Briefly, individual cerebral hemisphere areas were measured and segmented from T2w images and lesion area was measured and segmented from DW images. A normalizing factor (EF) was calculated as an average of the whole brain area/ipsilateral hemisphere area for each slice. Lesion area, from DW images was calculated as a percentage of the ipsilateral hemisphere and normalized by multiplying by EF, which gave hemispheric lesion area %(MRI%HLA).

Histology- Rats were sacrificed immediately after imaging and brains were sectioned into 1mm slices. Slices were incubated in 2,3,5-triphenyltetrazolium chloride (TTC) dissolved in PBS (1mg/ml) at 37.5°C for 15mins before fixation. Sections were scanned on a Canon desktop scanner. Images were analysed by manual segmentation using ImageJ. The Swanson Method was used to correct for cerebral enlargement and to calculate TTC%HLA [5].

RESULTS- High quality T2w and DW images were acquired for all animals (FIG 1). Pathological hallmarks of ischemia at 24 hours were observed on T2w, DW and TTC images. There was a significant difference in %HLA between the 30 and 60 minute cohorts, determined by both 1T MRI and histology (students t-test, $p<0.05$). %HLA was calculated from TTC and MRI images for each slice and compared using a linear regression model that showed a strong positive linear correlation between TTC%HLA and MRI%HLA (FIG 2) for both the 30 and 60 minute cohorts (R^2=0.70, 0.76, respectively). Furthermore, data point distribution in FIG 2 shows that benchtop MRI is sensitive to detect both small and large areas of focal ischemia.

DISCUSSION- In this study, we have shown for the first time that a 1T benchtop MRI system can be used to acquire robust images in a rat stroke model. Despite the low-field, contrast-to-noise was sufficiently high to acquire images that could differentiate between small and large ischemic insults. We have also demonstrated that MR image quality was sufficient to implement a novel method of lesion analysis that uses both T2w and DW data to account for cerebral swelling and produce quantitative measures of hemispheric lesion area. This novel analysis provided a strong positive correlation with gold-standard histology, thus validating it as a technique for preclinical stroke assessment in rats. To conclude, 1T benchtop MRI systems offer promise for low-cost, accurate measurement of lesion area in pre-clinical models of ischemic stroke.

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