Assessing Stroke-Induced Injury in Stroke-Prone Rats: MRI Thresholds of Tissue Abnormality

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Summary

Following stroke, magnetic resonance imaging (MRI) can be used to identify injured brain tissue. However, a threshold of tissue abnormality must be applied to accurately delineate the extent of the injury.

In rodent models of stroke, one study group have defined such a threshold, using Sprague-Dawley rats. This threshold may not be applicable to other rat strains, so we aim to establish a threshold in two other strains: the spontaneously hypertensive stroke-prone (SHRSP) and its reference, the normotensive Wistar Kyoto rat (WKY).

Following experimental stroke, the thresholds of tissue abnormality were calculated and applied to MR images to identify brain injury. The calculated thresholds were comparable between the SHRSP and WKY strains but the published threshold of tissue abnormality significantly underestimated the extent of injured tissue.

Therefore, we conclude that thresholds of tissue abnormality need to be defined for individual rat strains.

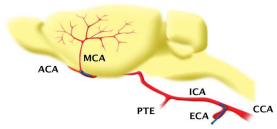

Figure 1. The Intraluminal Filament Model of middle cerebral artery occlusion (MCAO). Image depicts insertion of the filament via the external carotid artery and shows the territory supplied by the MCA.

Introduction

- In the acute phase following stroke, ischaemic injury can be identified with MRI using diffusion weighted imaging (DWI).
- DWI identifies restricted diffusion associated with cytotoxic oedema, characterised by a reduced apparent diffusion coefficient (ADC) value of brain water.
- Establishing ADC thresholds of abnormality that predict final infarct is essential in order to assess differences in the acute evolution of damage and the efficacy of potential interventions.
- To date, only one group has established ADC thresholds of abnormality following stroke using Sprague-Dawley (SD) rats[1]. The published ADC threshold value of 0.53 x 10^{-3}mm^2/sec may not be applicable to other rat strains due to differences in lesion evolution.
- Spontaneously hypertensive stroke-prone rats (SHRSP) were derived by selective breeding from normotensive Wistar Kyoto (WKY) rats. They progressively develop severe hypertension, are at increased risk of spontaneous stroke and have increased sensitivity to experimental stroke[2].
- SHRSP represents a clinically relevant model of stroke as hypertension is a well established risk factor in human stroke.

Aim

To establish an ADC threshold of abnormality that predicts final infarct in the clinically relevant spontaneously hypertensive stroke-prone rat (SHRSP) and its normotensive control, the Wistar Kyoto rat (WKY).

Methods

- Age matched adult male WKY (321±25g, mean systolic BP 133±7mmHg, n=8) and SHRSP (276±27g, mean systolic BP 187±12mmHg, n=7) were anaesthetised and ventilated with isoflurane (induced with 5% and maintained on 2-2.5%) in N_2O:O_2 (70:30).
- Permanent middle cerebral artery occlusion (**MCAO**) was induced using the intraluminal filament model (Figure 1).
- Animals were transferred to the MRI scanner, body temperature was maintained at 37±0.5℃ and blood samples taken ever y hour from a femoral artery cannula to control physiological parameters.
- **Ischaemic damage** was assessed with a Spin-Echo based Echo Planar DWI scan (matrix: 96 x 96, FOV: 25 x 25 mm, 8 x 1.5 mm slices)
- DWI scans were repeated every hour from 1 to 4 hours post MCAO.
- Femoral artery cannula was removed and animals were allowed to recover until 24 hours post MCAO.
- **Final Infarct** was assessed by T_2 weighted imaging (matrix: 256 x 256, FOV: 25 x 25 mm, 16 x 0.75mm slices) at 24 hours post MCAO.

Data Analysis

- Quantitative ADC maps were generated using Image J software.
- Final infarct volume was calculated by manually delineating the area of hyperintensity on T_2 weighted images at 24 hours post MCAO.
- Brain oedema was corrected for using published equations[3].
- ADC thresholds were adjusted until the ADC-derived lesion matched final infarct (measured by T_2 weighted imaging).
- Data are presented as mean±SD. Level of statistical significance defined as P≤0.05. All statistical tests are two-sample t tests unless otherwise stated.

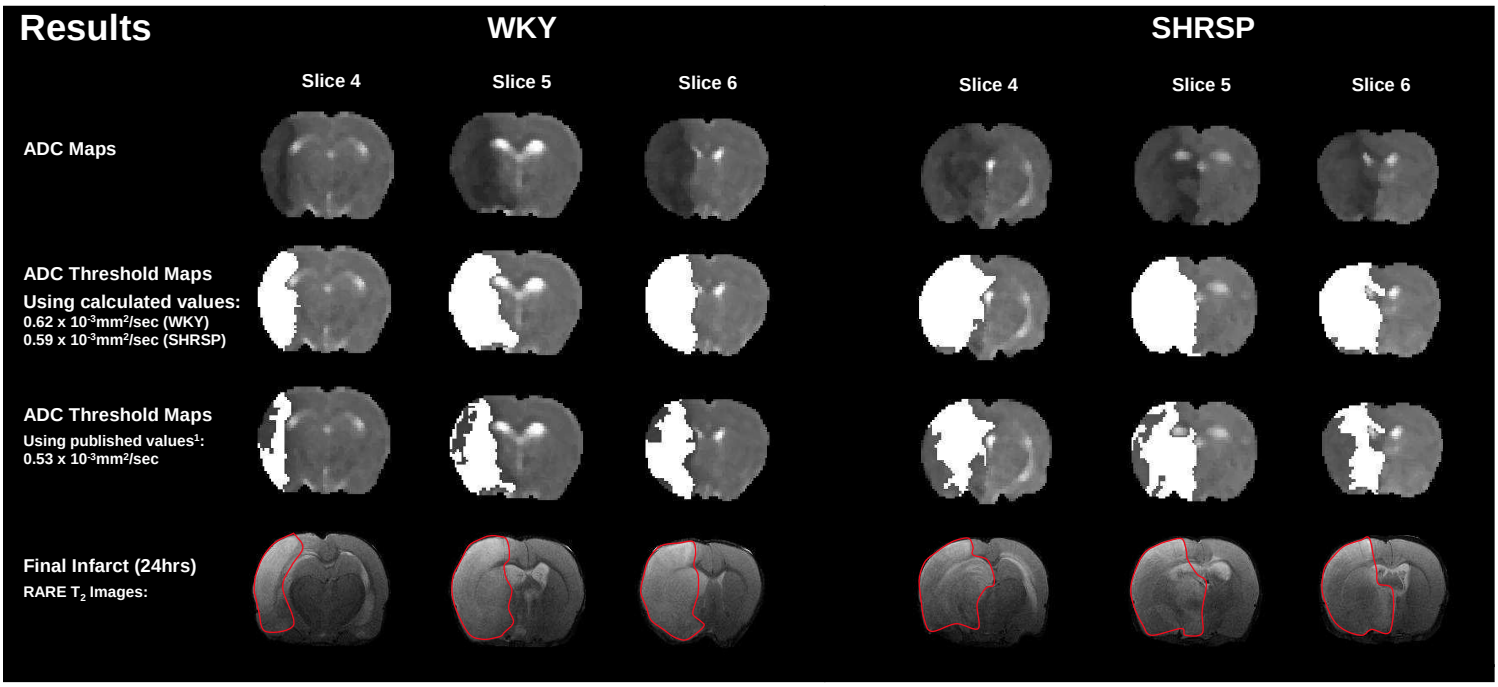

Figure 2. Mean calculated ADC thresholds for SHRSP, WKY and the published ADC threshold for SD rats applied to ADC scans to identify ischaemic injury at 4 hours post MCAO. Scans at three coronal levels are displayed in each row. Injured tissue is shown in white. Tissue injury defined from using calculated ADC thresholds matches well with final infarct on T_2 at 24 hours post stroke (outlined in red). Published ADC threshold underestimates ischaemic injury.

The calculated thresholds are:

WKY: ADC≤0.62±0.03 x 10^{-3}mm^2/sec

SHRSP: ADC≤0.59±0.03 x 10^{-3}mm^2/sec

- There was no significant difference in ADC thresholds between WKY and SHRSP (P=0.15), Figure 3.

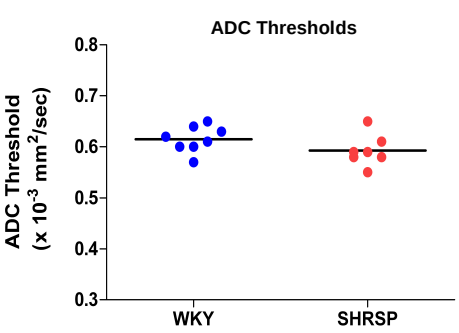

Figure 3. ADC thresholds derived in each rat from final infarct volume. Horizontal bar represents mean. Threshold values are comparable between WKY and SHRSP (P=0.15)

- Application of the calculated ADC thresholds shows that the volume of ischaemic injury is significantly greater in SHRSP than WKY at all time points following stroke (P<0.05), Figure 4.
- By 3hrs post-stroke, the ischaemic lesion has fully evolved in WKY, with no further growth in volume at the 4hr time point. By comparison, the ischaemic lesion continues to evolve until 4hrs post-stroke in SHRSP, Figure 4.

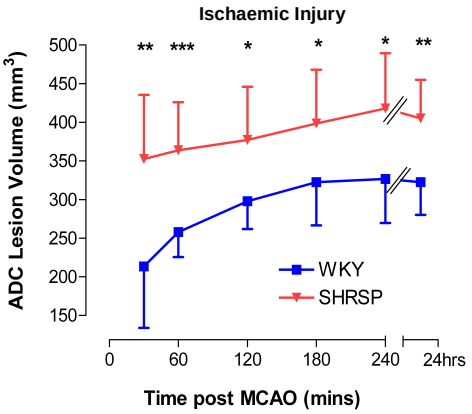

Figure 4. Temporal evolution of ischaemic injury following stroke. The volume of injured tissue increases with time in both strains (Repeated Measures ANOVA P<0.05). The volume of ischaemic injury and final T_2 infarct at 24 hours are significantly greater in SHRSP compared to WKY using the calculated ADC thresholds for each strain (*P≤0.05, **P≤0.01, ***P≤0.001).

- The final infarct at 24hrs is significantly larger in SHRSP than WKY (405±50mm^3 vs 322±43mm^3, P=0.004), Figure 4.
- The previously published ADC threshold value of 0.53 x 10^{-3} mm^2/sec[1], significantly underestimates the volume of injured tissue in both strains (P<0.05), Figure 2.

Conclusions

- The volume of injured tissue observed following MCAO is significantly greater in SHRSP compared to WKY at all time points, confirming the increased sensitivity to experimental stroke reported in the hypertensive strain.
- The calculated ADC thresholds are comparable between SHRSP & WKY and can be applied to identify ischaemic injury following experimental stroke.
- The published ADC threshold defined in Sprague-Dawley rats, significantly underestimates ischaemic injury in SHRSP and WKY.
- It is necessary to establish thresholds for each rodent strain to avoid over- or underestimation of ischaemically injured tissue and potentially salvageable penumbra following stroke.

References

{1} Meng X et al. Characterizing the diffusion/perfusion mismatch in experimental focal cerebral ischaemia. Annals of Neurology (2004) 55: 207-212.
{2} Coyle P & Jokelainen P.T. Differential outcome to middle cerebral artery occlusion in spontaneously hypertensive stroke-prone rats (SHRSP) and Wistar Kyoto (WKY) rats.Stroke (1983) 14: 605-611.
{3} Gerriets T et al. Noninvasive quantification of brain edema and the space-occupying effect in rat stroke models using magnetic resonance imaging. Stroke (2004) 35: 566-571.

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