

Infarct volume determined by acute ADC correlates neurological outcome in stroke mice

C-W. Chiang¹, T-H. Wu², J. H. Kim², and S-K. Song²

¹Chemistry, Washington University, St. Louis, MO, United States, ²Radiology, Washington University, St. Louis, MO, United States

Introduction

Rodent models have been employed to study human stroke, the third leading cause of death and disability in western countries. *In vivo* magnetic resonance (MR) imaging including advanced diffusion technology has been employed to examine infarcted tissues after stroke¹. The decreased apparent diffusion coefficient (ADC) has been demonstrated as a sensitive and reliable biomarker of cerebral ischemia²⁻³. In addition, there have been efforts to correlate MR findings with neurological behavior outcomes. Various behavior assessments have been employed including neurological scoring system⁴, rotor rod⁵, and methamphetamine-induced rotation test⁵. However, an accurate behavior test avoiding subjective ratings, significant training effect, or injection of medicine is still needed. Footprint analysis is a simple and sensitive measurement for gait analysis and has been applied to objectively evaluate the step cycle of mice^{6,7}. In this study, the gait analysis of middle cerebral artery occlusion (MCAO) mice was performed using a treadmill to examine the effect of lesion severity on neurological function. Acute DTI derived ADC delineated ischemic lesion clearly enabling quantification of the lesion volume and lesion ADC. The ADC defined lesion volume correlated well with injury severity, and neurological motor function.

Methods

Eighteen male, 8 – 12 weeks old, C57BL/6 mice weighing between 20 – 28 g underwent different degrees of stroke injuries (n = 6 for each group) by electrocoagulation of middle cerebral artery at the right side of the mouse brain. The injury severity was regulated by controlling energy of electrocoagulation at 0 (craniotomy), 1.4 (mild), and 1.8 mJ (moderate). *In vivo* DTI was immediately performed on a 4.7 T magnet utilizing a standard spin-echo diffusion-weighted sequence. All images were obtained with acquisition parameters of TR 1.7 sec, TE 50 ms, Δ 25 ms, δ 8 ms, NEX 4, slice thickness 0.5 mm, field-of-view 3.0 cm \times 3.0 cm, data matrix 256 \times 256 (zero filled to 512 \times 512), (Gx,Gy,Gz) = (1,1,0), (1,0,1), (0,1,1), (-1,1,0), (0,-1,1), and (1,0,-1), and b = 0 and 0.768 ms/ μ m². The locomotor function (left foot base) of the mice was assessed using the treadmill with gait analysis software TreadScanTM 2.0 (Clever Sys. Inc., VA, USA). The evaluation was performed at a speed of 15 cm/s without pre-training. The statistical analysis, two sample student *t* test and Person-product linear correlation, was performed with Origin 7.5 SR2 v7.5817 (Origin Lab Co., MA, USA).

Results and Discussion

In acute stage, i.e., at 3 hours after stroke injury, the tensor derived ADC maps clearly revealed the infarcted cortex as the hypointense region on ADC maps in stroke mice. The region of interest (ROI) analysis based on the ADC contrast between the infarct and the non-injured cortex was used to quantify the total lesion volume (Fig. 1a). As the energy deposition elevated to increase the injury severity, the infarct volume increased significantly (Fig. 1b). Although there was a statistically significant difference between the stroke mice and the control, the ADC value of the lesion area among the stroke groups were not statistically significantly different (Fig. 1c). The result suggests that the lesion volume determined using the ADC correlated well with the injury severity intended by modulating the deposited coagulation energy.

The left and right foot base, the distance between mid-points of strides of the pairs of feet, was assessed using mouse treadmill as neurological functional measure. Prior to any experimental procedure, there was no foot base difference among all mice as expected. Since we did the surgery on the right side of the mouse brain and the infarct covers the motor and sensory cortex, we expected a neurological deficit to the left side. Indeed, animal with ischemia showed shortened left foot base compared to naïve and craniotomy group. A linear correlation between the volume of acute infarction and left foot base at day 3 is observed (Fig. 2). There was no observable foot base change for right lateral limbs (data not shown).

Conclusion

As previously reported¹⁻⁵, *in vivo* diffusion examination of brain tissue sensitively revealed ischemic lesion. Our results indicated that the total ADC determined volume of infarction reflected stroke injury severity better than ADC itself. The acutely quantified infarct volume correlated with the locomotor function deficit assessed using gait analysis of the mouse treadmill.

References

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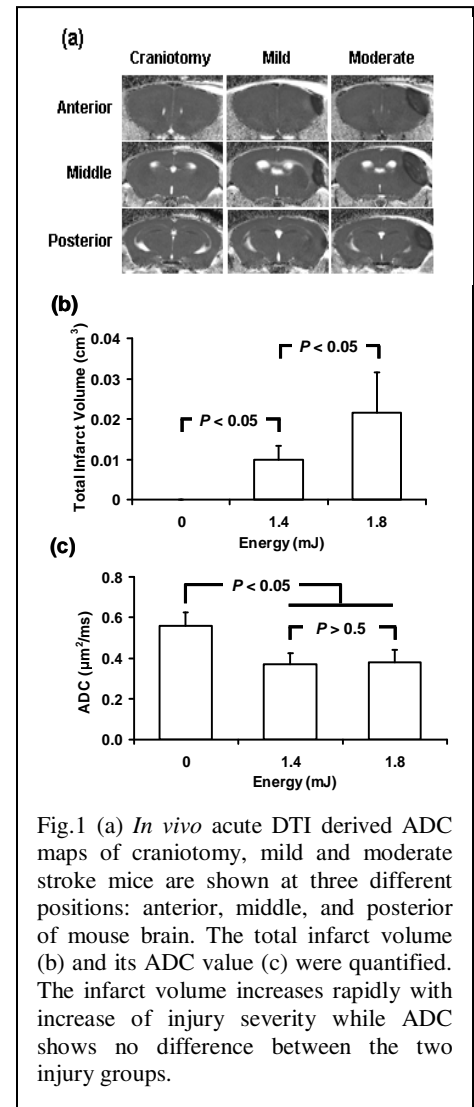


Fig.1 (a) *In vivo* acute DTI derived ADC maps of craniotomy, mild and moderate stroke mice are shown at three different positions: anterior, middle, and posterior of mouse brain. The total infarct volume (b) and its ADC value (c) were quantified. The infarct volume increases rapidly with increase of injury severity while ADC shows no difference between the two injury groups.

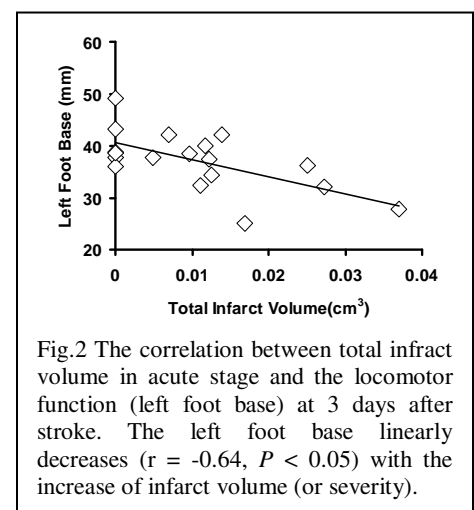


Fig.2 The correlation between total infarct volume in acute stage and the locomotor function (left foot base) at 3 days after stroke. The left foot base linearly decreases ($r = -0.64$, $P < 0.05$) with the increase of infarct volume (or severity).