Magnetic Resonance Imaging as an In-vivo Tool for Evaluating Efficacy of Brain Edema Prevention Therapy in a Rat Stroke Model

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Current treatment strategies for acute stroke involve clot thrombolysis and reperfusion of the ischemic penumbra. However, rapid reperfusion can potentially lead to other downstream deleterious effects including blood-brain barrier (BBB) degradation and subsequent edema. Research focused on understanding structural and functional pathologic changes occurring from the onset of stroke to the hours and days following stroke has benefited from the use of various MRI techniques. T1/T2-weighted, diffusion, and perfusion parameters provide in vivo surrogates of BBB integrity, water/cell content, edema and vascularity. While numerous studies have used these MR endpoints for studying stroke pathology, there has been limited use of MR parameters as in vivo markers of novel treatment efficacy. Here, Gd-enhanced T1-w and T2-w MR data were used to verify the efficacy of pre- and post-infarct treatment with a novel KDR kinase inhibitor (KDRi) known to reduce vascular permeability and therefore, BBB leakage. In line with previously published ex-vivo data (1), in-vivo MRI results suggest efficacy of KDRi treatment in reducing BBB leakage and edema formation, as indicated by tissue water content.

Methods: Rats were subject to 90 min transient middle cerebral artery occlusion (tMCAO). KDRi administration at a dose sufficient to produce 24hr plasma levels above the IC50 occurred either 0.5 hr prior to occlusion (pre-tx) (n=9) or 2hr post occlusion (post-tx) (n=5). Control group (n=9) received vehicle. MRI experiments were conducted on a Bruker 7T/30 Biospec with a 72mm volume transmit coil and a surface quadrature receive coil. Rats were anesthetized with isoflurane via a nose cone and imaging was conducted approximately 24hours after the start of the MCAO. T1-w DCE-MRI was performed with the following parameters: FLASH, 8slices, 2mm slice, 32x32mm FOV, 128x80 matrix, TR/TE= 100/4ms. Approximately 19 images were acquired for determination of baseline signal followed by an iv bolus of 0.3mmol/kg of Gd-DPTA. BBB permeability was defined as the difference between the ispsilateral and contralateral AUCs normalized to the AUC of the contralateral hemisphere. Water content was assessed by multiple measures. T2 maps were acquired as follows: multi-echo RARE, 8slices, 2mm sl. thickness, 32x32mm FOV, 128x80 matrix, TR 3s, TEs 15.6, 46.9, 78.2, 109.5, 140.8ms, rare factor 2. Average T2 and T2 histogram analysis were performed for T2 intensities within the lesion, with longer T2s indicating higher water content. Edema volumes were calculated as the percent of the entire lesioned hemisphere normalized to the contralateral hemisphere as described in Gerriets et al (2).

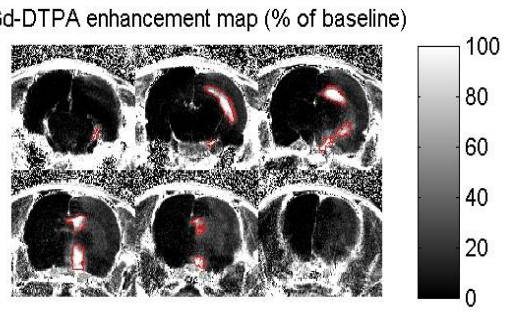

Figure 1. Maps of increased % intensity derived from Gd-DPTA enhanced multislice images. Areas in red indicate an intensity increase above two standard deviations of the signal from the contralateral hemisphere.

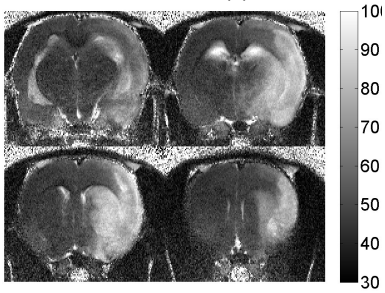

Figure 2. T2 map used for histogram analysis to determine water content.

	control n=9	SE	Pre-tx n=9	SE	p	Post-tx n=5	SE	p
Gd-AUC difference (%contra)	77.0%	16.9%	46.1%	9.4%	0.069	26.8%	11.3%	**0.015**
Edema Volume (% hemi)	14.4%	1.8%	10.0%	2.1%	0.069	10.3%	1.5%	0.055
Average T2 (ms)	72.08	1.38	68.37	2.03	0.083	69.10	1.62	0.093
25 percentile T2 (ms)	65.82	1.07	61.50	1.42	**0.02**	61.50	1.57	**0.02**

Table 1. Results for vehicle treated control group, pre-treatment (pre-tx) with KDRi at 0.5 hr prior to occlusion, and post-treatment with KDRi 2 hr post occlusion. SE= standard error. P values refer to pre-tx vs control.

Results/Conclusion: Post-treatment with a novel KDR inhibitor produced a significant reduction in Gd-DPTA enhancement AUC compared to vehicle controls (Table 1), indicating reduced BBB leakage at 24hr following acute ischemia/reperfusion in compound treated animals. Pre-treatment with this compound also produced a reduction in Gd-DPTA enhancement AUC compared to vehicle controls, but not significant. Edema volumes in KDRi inhibitor- treated animals were also reduced compared to vehicle controls, though this measurement was not statistically significant for pre- or post- treatment (Table 1). Water content as assessed by T2 maps trended to shorter average T2 with treatment, but did not achieve statistical significance between groups, possibly due to the heterogeneity within the lesions. However, histogram analysis revealed a similar trend for the 75th percentile, and statistically significant decreases of the 25th percentile in the treatment groups compared to vehicle controls (Table 1). Difference between treatment groups, however, was not significant. In a separate cohort, ex vivo analysis of % water content in the lesion (data not shown) showed a significant decrease in the pre-treatment group and a nearly significant decrease in the post-treatment group, supporting our T2 data as a surrogate for water content (1).

Vascular endothelial growth factor (VEGF) may modulate vascular permeability and subsequent plasma extravasation/edema following stroke (1). Pharmacologic inhibition of the VEGF pathway via the KDR kinase receptor might prevent these permeability changes and decrease edema formation. In vivo tools for verification of these hypotheses are lacking, however this study suggests MR is a good candidate for assessment of such pharmacologic interventions. Our results, in agreement with the ex vivo data, suggest that KDR kinase inhibition is efficacious in reducing tissue water content in the ischemic hemisphere. This water content reduction is a probable result of a compound-induced reduction in BBB leakage, as shown by the Gd-enhanced MR data. In conclusion, this study demonstrates the utility of MR to non-invasively evaluate novel compound efficacy for potential application in stroke management.

References: 1.Foster et al, Neuro Res 63;10-16, 2009; 2. Gerriets et al, Stroke 35:566-571, 2004.