Development of a Porcine Middle Cerebral Artery Occlusion Stroke Model and Stroke Characterization with Quantitative MRI Techniques.

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Introduction: Rodent studies using induced pluripotent stem cell (iPSC) derived neural progenitors (or iNPs) have shown potential as a robust, autologous regenerative cell therapy to replace damaged brain tissue. However, rodent stoke models have significant limitations when used in conducting imaging and therapeutic trials, as well in studying certain mechanisms of disease and treatment responses.[1,2] For translation to humans, a large animal model is more desirable for testing of therapies. With an interest in pursuing stem therapeutic options for stroke, we undertook the development of an economical large animal middle cerebral artery occlusion (MCAO) model that would allow *in vivo* serial quantification of stroke parameters that could be used to assess novel therapeutic strategies. This works report the quantitative MRI assessment of the pig MCAO stroke model utilized in a study of a novel iNP therapeutic.

Methods: Ten adult male Landrace pigs underwent surgically induced MCAO by occluding the branches of the superficial temporal artery and associated vein, using high frequency bipolar electrocautery forceps. Following stroke induction, each pig underwent a MRI examination at 24 hours, as well as at 1, 4 & 12 weeks following stroke induction, using a 1.5T Siemens Symphony with TIM, with the anesthetized pigs positioned in a 12-channel head and neck coil. This abstract is focused on the 24-hour post-stroke induction. The imaging protocol consisted of T2, T2 FLAIR, DWI, DTI, PWI, CSI spectroscopy and pre- and post-contrast T1 images. To quantitate the stroke induction, ADC values in the affected portion of the cerebral hemispheres were measured. White matter involvement was evaluated by measuring fractional anisotropy (FA) within the stroke territory. Molecular alterations, specifically NAA, Cr and Cho values, were measured as indicators of neural cell damage, metabolism and cellularity, respectively. Normal values were established in the contralateral cerebral hemisphere. ADC values, FA and spectroscopy measures were evaluated in triplicate. Comparisons were assessed using a Student's T-test, with significance set at $p<0.05$.

Results: All pigs could be positioned within the head and neck coil. This configuration had sufficient signal-to-noise to maintain anatomic resolution at or less than 0.5 x 0.5 x 3 mm^3. The infarct region and extent of the tissue damage was well delineated on DWI and T2 images respectively. The mean stroke volume was 7.83cc (range 3.09 – 15.27cc). The mean volume on T2 images, presumably including the penumbra of the stroke, was 9.93cc (ranging from 5.64 – 15.66cc). Significant decreases in the stroke ADC were measured, indicating successful induction of an ischemic area in this pig breed. Mean stroke ADC value was 495.27 mm/s (SD=63.23), whereas the contralateral hemisphere mean ADC value was 719.58 mm/s (SD=35.01), with $p<0.01$. This change involved both gray and white matter regions. The FA values in the white matter of the stroke area had a mean of 320.1 (SD=54.8) and were significantly different ($p<0.001$) from the identical white matter region in the normal hemispheres (mean = 553.75; SD=97.9). There was also significance difference in all spectroscopy metabolites and their ratios, except for Cr (see Table 1). In the PWI of all pigs, low rCBV/CBF was observed in the infarct cerebral hemisphere, compared to the control hemisphere.

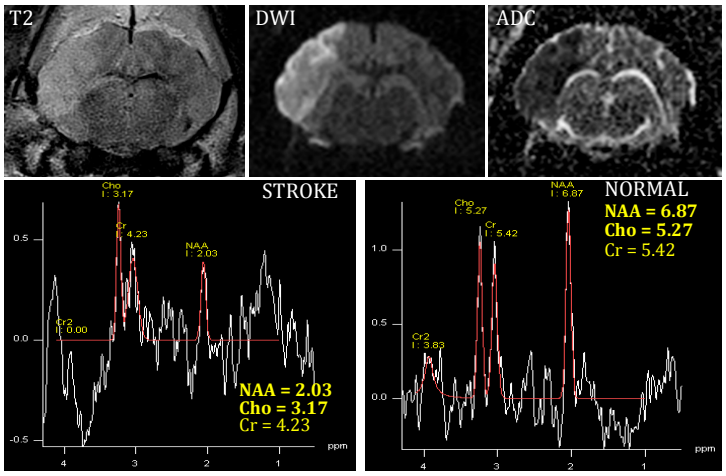

Table 1: Stroke neural metabolic marker values (with Normal)

Metabolite	NAA	Cho	Cr	NAA/Cho	NAA/Cr
Mean	0.81 (4.08)	2.53 (5.52)	3.28 (4.18)	0.33 (0.85)	0.48 (1.15)
Std Dev	0.33 (1.30)	0.69 (1.82)	2.62 (1.88)	0.13 (0.42)	0.52 (0.46)
p value	0.002	0.013	0.466	0.039	0.029

Discussion: With greater interest in serial and long-term studies of this large animal model, the Landrace pig was considered despite concern for its larger size. Our MRI results are similar to the prior study,[4] indicating that a Landrace pig MCAO model is feasible, and can be studied at 1.5T. Significant quantitative differences in key MR stroke parameters (ie. ADC value, white matter and the molecular environment) were defined in a *limited* number of pigs. As it is a more economical model, more post-stroke MR imaging time points can be evaluated, which will enhance our understanding of the evolution of a stroke and/or its response to a selected therapeutic agent.

Conclusion: This pilot study indicates that it is feasible to detect differences in *in vivo* biochemical markers of an ischemic event in a large animal model that has similar gyrification, brain size and gray-white matter composition to humans. Therefore, this model can be used to test novel stroke therapies, such as stem cells.

References:

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