ASSESSMENT OF PHARMACOLOGICALLY INDUCED HYPOTHERMIA IN A RODENT MODEL OF FOCAL CEREBRAL ISCHEMIA USING DIFFUSION TENSOR IMAGING

Silun Wang[1], Xiaohuan Gu[2], Ramesh Paudyal[1], Shan Ping Yu[2], and Xiaodong Zhang[1,3]

[1]YERKES IMAGING CENTER, Emory University, Atlanta, GA, United States, [2]Department of Anesthesiology and Department of Neurology, Emory University School of Medicine, GA, United States, [3]Division of Neuropharmacology and Neurologic Diseases, Yerkes National Primate Research Center, GA, United States

Target audience: MRI scientists, Radiologists and experimental neurologists.

Purpose: Therapeutic hypothermia reduces the energy demands of neuronal activity and attenuates free radical levels which help to protect the brain from ischemia insult[1]. Quantitative analysis of diffusion indices has shown promise to evaluate pathological changes within stroke lesion[2-5]. In the present study, DTI was utilized to assess pharmacologically induced hypothermia treatment response in a stroke model of mice.

Methods: ***Animal model preparation:*** Focal cerebral ischemic stroke was induced by right middle cerebral artery (MCA) occlusion in adult male C57BL/6 mice (25-40 g). Animals in the control group were injected with saline after stroke, and their body temperature was maintained at 36–37°C in a humidity-controlled incubator (Thermocare, NV, USA) until MRI scanning. ***Hypothermia treatment:*** Animals were subjected to ABS-201 injection, a novel neurotensin analog, to influence their body temperature. Briefly, ABS-201 was dissolved in saline and injected intraperitoneally with the bolus injection (2 mg/kg) at 30 min after CCA reperfusion, followed by additional injections at half of the initial dose (1.0 mg/kg). The interval between the following injections was around 1.5 hrs, with adjustments made in order to keep a constant mild hypothermia (33–35°C). Body temperature was monitored using a rectal probe every 30 minutes after injection. ***MRI scanning and data analysis:*** Mice were evaluated by MRI scanning at first day (1hr, 2hrs and 3hrs) and 24 hrs post-surgery using 7T MR scanner (Bruker, Germany). Whole brain T2-Weighted images (T2WI) and DTI were performed using the following parameters: T2WI: TR/TE=1000ms/50ms, FOV = 30mm^2, thickness= 0.5mm, acquisition matrix = 128 x 128; DTI will be acquired using a multiple-slice, spin-echo sequence with TR/TE =3000ms/32ms, FOV = 20mm x 20 mm, thickness= 0.5mm, data matrix = 96 x 96, in-plane resolution = 0.33 × 0.33 mm^2. Six gradient directions with b value =0 and 1000 s/mm^2, respectively. ADC maps were derived for quantitatively analyze by using DTIstudio v2.4 and analyzed by Image J (NIH, U.S). **Histology:** Rats were sacrificed for histological evaluation immediately after their last MRI scanning (48 hrs post surgery). A TUNEL assay kit (DeadEnd Fluorometric TUNEL system; Promega, Madison, WI, USA) was used to assess cell death. NeuN (1:300; Millipore, Billerica, MA, USA) and Hoechst 33342 (1:20000; Molecular Probes) were used to identify nuclear of Neuron and all nuclei. Cell count was performed following the principles of design based stereology and quantitatively analyzed.

Results: ***ABS-201 induced hypothermia:*** At the dosage of 1.5mg/kg, ABS-201 induced core body temperature dropped below 35°C within 15 min after injection and maintained for 60 min without detectable shivering. All animals were awake during the hypothermia treatment. ***Comparison of stroke volume between treatment and control groups***: The in *vivo* MRI findings of ischemic lesions in the hypothermia treatment and vehicle group are illustrated in Fig 1. In both groups, ischemic region was observed at cortex as early as 0.5 hr post occlusion. Hypothermia treatment shows obvious neuroprotective effects in regard to stroke volume. The stroke volumes of vehicle group were significantly larger than those in ABS-210 treated group at 1 hr (57.4 mm^3 vs. 4.6 mm^3, p=0.01), 2 hrs (65.9 mm^3 vs. 7.3mm^3, p=0.05), 3 hrs (79.8 mm^3 vs. 7.2mm^3, p=0.06) and 24 hr (201.2 mm^3 vs. 39.6 mm^3, p=0.03) post occlusion. Infarct volumes at 24 hr after stroke reduced by 80% in the hypothermia treatment group compared to the control group. ***Quantitative analysis of ADC values in stroke lesion***: In both groups, ADC values were found gradually decreased in the ischemic lesion from 1 hr to 24 hrs post occlusion. However, ANOVA test did not show significant differences. In addition, slightly lower ADC values were observed in the control groups compared to hypothermia treatment group. No significant differences were found among all time points (Fig 2). ***Histology:*** The immunohistological results of treatment and vehicle mice at 48 hours post surgery were shown in Fig 3. Nuclei of all cells were visualized with Hoechst staining (blue); Neuronal nuclear were identified by NeuN staining (red) and apoptotic cells were identified by TUNEL staining (green). TUNEL-positive cells were fewer in ABS-201-treated group (F) compared with the vehicle group (E). TUNEL (green) and NeuN (red) double-positive cells represent neuronal cell death in the penumbra. Total cell death (TUNEL/Hoechst positive) and neuronal cell death (TUNEL/NeuN positive) in ABS-201-treated group (H) were significantly fewer than vehicle group at 24 hours post occlusion (G).

Discussion: (1). Comparing with physical cooling, ABS-201 can cause 2~5°C body temperature reduction within 60 minutes and avoid complications in physical cooling such as shivering and vasoconstriction responses. (2). Significantly smaller stroke lesions were demonstrated in acute stroke (1-3 hrs) and 24 hrs post occlusion in the hypothermia treatment group compared to vehicle group. (3). ADC reduction in both treatment and control group indicated the cytotoxic edema. Mild ADC reduction in hypothermia group may reflect less cellular injury compared to vehicle group. (4). ABS-201 induced hypothermia shows promise neuroprotective effects in the ischemia.

Conclusion: Our results support the use of diffusion indices in ischemic lesion as biomarkers to non-invasively monitor the ischemia-induced injury as well as to evaluate hypothermia treatment response. Also, the specific hypothermia treatment with ABS-201 may be effective to reduce the stroke lesion volume.

References : 1. Jiang et al, Neuroimage, 2006;32:1080-1089; 2. Bihel et al, Stroke,2011;42:1412-1419; 3. Pitkonen et al, Brain Research, 2012;103-110; 4. Qiu et al, Neurorehabilitation & Neural Repair, (2011);25(3) 275-284; 5. Wang et al., Stroke 2008; 39: 2348-2353. 6. Choi et al., The FASEB journal, 2012;26: 2799-810.

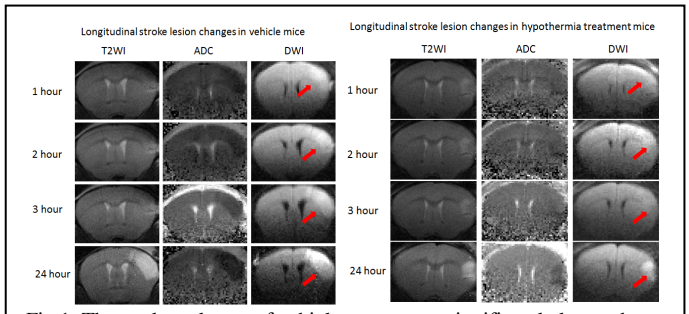

Fig 1: The stroke volumes of vehicle group were significantly larger than hypothermia treated group at 1 hr, 2 hrs , 3 hrs and 24 hr post occlusion. Infarct volumes at 24 hr after stroke reduced by 80% in the hypothermia treatment group compare to the control group.

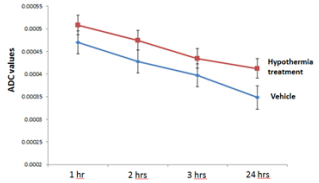

Fig 2. In both groups, ADC values were found gradually decreased in the ischemic lesion from 1 hr to 24 hrs post occlusion. Slightly lower ADC values were observed in the vehicle groups.

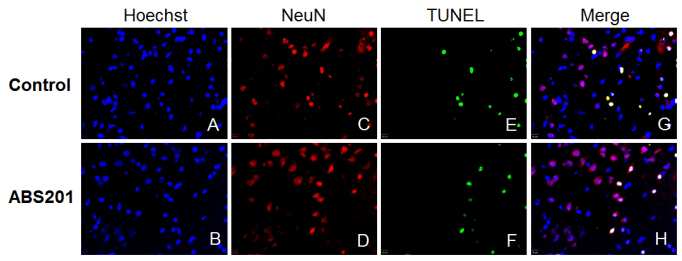

Fig 3: Immunohistological evaluation: Total cell death (TUNEL/Hoechst positive) and neuronal cell death (TUNEL/NeuN positive) in ABS-201-treated group (H) were significantly fewer than vehicle group at 24 hours post occlusion (G).