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

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Target audience: MRI scientists, Radiologists and experimental neurologists.

Purpose: Diffusion tensor imaging (DTI) allows the non-invasive measurement of in vivo 3D diffusion of water molecules in tissue and has been demonstrated to be a robust tool to access myelination and axonal integrity. Quantitative analysis of DTI indices has shown promise to evaluate pathological changes in white matter with stroke lesion[1-5]. Therapeutic hypothermia reduces the energy demands of neuronal activity and attenuates free radical levels which help to protect the brain from ischemia insults[2]. In the present study, we hypothesized that DTI indices could be applied as imaging biomarkers to access the response of pharmacologically induced hypothermia treatment in an ischemic 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 (x35-40 g). Briefly, the distal branches of right MCA was permanently ligated, accompanied by transient bilateral common carotid artery (CCA) ligations for 7 minutes. Animals in the control group were administrated with saline after stroke, and their body temperature was maintained at 36–37°C in a humidity-controlled incubator until MRI scanning. ***Hypothermia treatment:*** Animals were administrated with ABS-201, a novel neurotensin analog (NTR), to influence body temperature[6]. 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 (1.5 mg/kg). The interval between the injections was around 1.5 h, with adjustments made in order to keep a constant mild hypothermia (33–34°C). Body temperature was monitored with a rectal probe every 15 min after injection. All animals were awake during the hypothermia treatment. ***MRI scanning and data analysis:*** Mice were scanned at 4 and 24 hour post-surgery using a Bruker 7T scanner with a quadrature volume coil. T2-weighted images (T2WI) and DTI were performed using following imaging parameters: T2WI: TR/TE=1000ms/50ms, FOV = 30mm^2, thickness= 0.5mm, acquisition matrix = 128 x 128; DTI will be acquired using a spin-echo sequence with TR/TE =3000ms/32ms, FOV = 30mm x 30 mm , thickness= 0.5mm, data matrix = 96 x 96, in-plane resolution = 0.33 × 0.33 mm^2. 6 gradient directions withb value =0, 1000 s/mm^2. FA, axial and radial diffusivities maps were derived for quantitatively analyze by using DTIstudio v2.4. ROIs including external capsule (EC), fornix, cerebral peduncle (CP), anterior commissure (AC) and optical tracts (OT) were selected for data analysis using Image J (Fig 1). Paired t-test was used to detect statistical differences of DTI indices between ipsilateral/contralateral white matter tracts.

Results: ***ABS-201 induced hypothermia:*** ABS-201 treatment induced core body temperature reduction within 15 minutes after injection and the hypothermic effect was maintained for 6 hrs without detectable shivering. ***Comparison of stroke volume between treatment and control groups***: Infarct volumes at 24 hour after stroke reduced by 43% in the hypothermia treatment group compared to the control group (3.5 ±2.4 mm^3 vs 4.7± 2.3 mm^3). ***DTI indices in multiple white matter tracts*** (Fig 2): In control group, FA values were found significantly decreased in the ipsilateral EC and fornix compared to that in the contralateral side (both p<0.05). In addition, significantly lower axial diffusivity was observed in the ipsilateral EC and fornix (p=0.03 and p=0.02, respectively). In hypothermia treatment group, lower FA and axial diffusivity values in the ipsilateral EC and fornix were observed visually. However, the differences did not reach significant differences. In addition, DTI indice values in CP, AC and OT exhibited no difference in both control and treatment groups.

Discussion: (1). Comparing with physical cooling, ABS-201 can reduce body temperature for 2~5°C within 60 minutes and without complications such as shivering and vasoconstriction responses usually observed in physical cooling. (2). Significantly decreased FA and axial diffusivity at 24 hours post occlusion in control group indicates myelin damage or axonal degeneration in the white matter due to ischemic insults. In contrast, FA reduction is observed in treatment group also but not significant (3). Selective white matter injury may be due to hypoperfusion and unbalanced blood supply as EC and fornix have least collateral circulation. (4). ABS-201 shows promise neuroprotective effects in the ischemia induced white matter injury.

Conclusion: Our results support the use of DTI indices as biomarkers in white matter to non-invasively monitor the ischemia induced injury as well as to evaluate hypothermia treatment response. The specific hypothermia treatment with ABS-201 may be effective to reduce the stroke lesion volume and protect the white matter fibers, which could play a critical role in the functional recovery due to brain plasticity after stroke.

References : 1. Bihel et al, Stroke,2011;42:1412-1419; 2. Jiang et al, Neuroimage, 2006;32:1080-1089; 3. Pitkonen et al, Brain Research, 2012;103-110; 4. Qiu et al, Neurorehabilitation & Neural Repair, 2011;25: 275-284; 5. Wang et al., Stroke 2008; 39: 2348-2353. 6. Choi et al., The FASEB Journal, 2012; 26: 2799-2810.

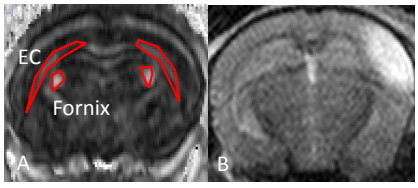

Fig 1 (A-B): Two ROIs including external capsule (EC) and fornix are illustrated in FA map (A) and T2-weighted image with stroke lesion (B).

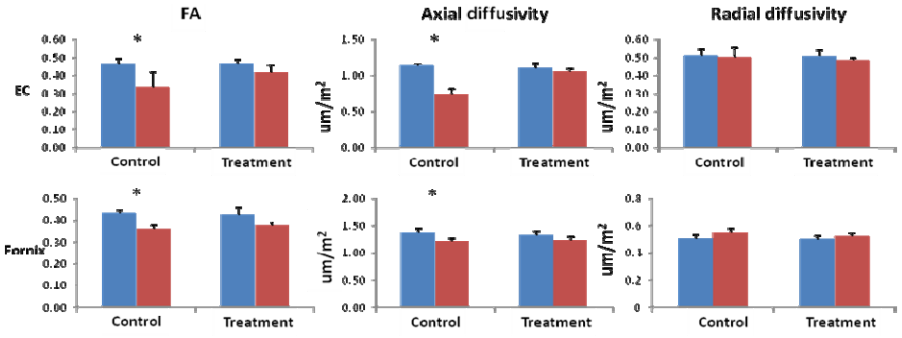

Fig 2: Quantitative analysis of DTI indices at ipsilateral (red bar)/contralateral (blue bar) EC and fornix at 24 hours post ischemia insults. Significantly lower FA and axial diffusivity were only demonstrated in control group but not in hypothermia treatment group. * indicates p<0.05.