Sabah Khan, Brain Disord Ther 2015, 4:4
http://dx.doi.org/10.4172/2168-975X.S1.002

OMICS International
conferenceseries.com

Global Summit on Stroke

August 03-05, 2015 Birmingham, UK

Cellular damage and changes in glutamate transporters in a murine model of ischemic stroke

Sabah Khan
University of Leeds, United Kingdom

Ischemic stroke or cerebral ischemia is a leading cause of death and adult disability but there is no effective treatment for this devastating disease. It is, therefore, important to understand stroke induced cellular damage and biochemical changes to find new potential targets for treatment of this disease. Glutamate plays a key role in the pathophysiology of stroke1 and glutamate levels in the brain are regulated by both plasma and vesicular glutamate transporters2. We have previously shown that the levels of specific plasma glutamate transporters are reduced following middle cerebral artery occlusion3. Recently we have optimised a murine model of cerebral ischemia induced by bilateral common carotid artery occlusion (BCCAO) in our laboratory. In the present study we have examined cellular damage and the changes in glutamate transporters (EAAT2, VGluT1 & VGluT2) following cerebral ischemia using western blotting and immunohistochemistry.

All experiments were performed on 10- 12 week old (26–30 g) C57BL/6 mice (Harlan-Olac, Bicester, UK) under UK Home Office License. Cerebral ischemia was induced in mice using arterial clips under inhalation anaesthesia with isoflurane for 15 minutes and sham-operated mice underwent the same procedure except that the carotid arteries were not occluded. Following 3-7days reperfusion time the mice were killed by scheduled 1 and the brains removed. The brain samples taken from both sides the cortex or the hippocampus were homogenised and protein was extracted using SDS-PAGE sample buffer. Proteins were transferred to polyvinylidene difluoride membrane (Amersham, GE Healthcare, UK) and probed with primary antibodies (GLTt-1, GLAST, VGluT1- & VGluT2,) followed by horse- radish peroxidase-conjugated anti-rabbit or anti-mouse IgG . The bound antibodies were visualised by using a Laser 3000 chemiluminescence detection system (Amersham Biosciences, Amersham, UK). Densitometry analysis in relation to total protein was performed using Image quantification software. The result showed consistent reduction in western blot of GLT-1, VGluT-1 and VGluT-2 as compared to β-actin in BCCAO animals. For immunohistochemistry the mice were re-anesthetised with isoflurane by inhalation and perfused with 4% paraformaldehyde. Brain sections (40-50um) were cut and processed for immunohistochemistry using specific antibodies (Neun, GFAP, GS, Glt-1, VGluT1- & VGluT2,) and analysed using an AxioImager Z.1 epifluorescence microscope (Carl Zeiss, Welwyn Garden City, UK).

The results suggest that vesicular glutamate transporters VGLUT1 and 2 along with specific plasma glutamate transporters may be potential targets for the prevention of excitotoxicity induced by ischemic stroke.

umssk@leeds.ac.uk

Notes: