ANTI-NOGO-A IMMUNOTHERAPY IS A POTENTIAL BREAKTHROUGH IN MEDICINE FOR POST-STROKE PATIENTS

Several studies have shown that antibodies that bind to the Nogo-A protein impede its ability to inhibit neuronal remodeling after injury to the central nervous system (Cheatwood et al., 2008a,b).

It is unclear, however, what exact mechanism allows functional recovery and anatomical reorganization after stroke to become enhanced, and the primary location of anti-Nogo-A antibody action is still unknown.

The main purpose of this experiment was to describe the expression of Nogo-A mRNA by astrocytes in the cerebral cortex of the post-stroke rat. We used an in vivo stroke model to determine the amount of astrocytic Nogo-A mRNA expression at several post-stroke time points.

INTRODUCTION

Anti-Nogo-A immunotherapy is a potential breakthrough in medicine for post-stroke patients. Several studies have shown that antibodies that bind to the Nogo-A protein impede its ability to inhibit neuronal remodeling after injury to the central nervous system (Cheatwood et al., 2008a,b).

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METHODS

Adult male Long-Evans Hooded rats underwent permanent unilateral middle cerebral artery occlusion (MCAO). Rats were sacrificed and perfused at 1, 3, 7, 14, or 28 days after stroke (a normal control group was also included). Brains were subsequently flash frozen and cut at 50µm on a cryostat. We then performed fluorescent in situ hybridization to label Nogo-A mRNA, and coupled it with fluorescent anti-GFAP immunohistochemistry to identify astrocytes in the same sections.

To quantify the Nogo-A immunofluorescence, the sections were photographed on a Leica DM 4500 B microscope using a Leica DFC 340 FX camera. The same settings were applied to the microscope for each image. Astrocyte cells expressing glial fibrillary acid protein (GFAP) were identified by green fluorescence and Nogo-A mRNA expression was identified by red fluorescence.

The images were exported to Image J software in split gray-scale channels to process and analyze the data. Astrocytes were identified on each brain section individually by the anti-GFAP fluorescence with a luminance of 35 or higher. These regions were then opened on the red channel of that image to measure the amount of Nogo-A mRNA at each of the selected locations.

Statistical analyses were performed and graphs were made using GraphPad Prism 5 software.

CONCLUSIONS

- No significant difference in the amount of astrocyte Nogo-A expression was evident at any time point examined (1, 3, 7, 14, or 27 days) post stroke compared to a normal, uninjured brain.

- Our results indicate a disconnect between Nogo-A mRNA and protein regulation after stroke, and suggest that interfering with Nogo-A protein translation from mRNA (i.e. siRNA) may be effective in limiting the post-stroke increase in Nogo-A protein in astrocytes.

LITERATURE CITED