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Proliferation in the human ipsilateral subventricular zone after ischemic stroke

J. Martí-Fàbregas, M. Romaguera-Ros, U. Gómez-Pinedo, S. Martínez-Ramírez, E. Jiménez-Xarrie, R. Marín, RN J.-L. Martí-Vilalta, J.-M. García-Verdugo.

Department of Neurology, Hospital de la Santa Creu i Sant Pau, Barcelona, Laboratorio de Morfología Celular, Centro de Investigación Príncipe Felipe, Valencia, Spain.

Correspondence : Dr. Joan Martí-Fàbregas, Department of Neurology, Hospital de la Santa Creu i Sant Pau, Avda. Sant Antoni Maria Claret, 167, 08025 Barcelona, Spain; Email : jmarti@santpau.cat

Deepali Mathur

810, University City Blvd, Apartment No. 5, Blacksburg, Virginia, USA

Background

Stroke is the leading cause of death and disability in industrialized nations and is projected to overtake coronary heart disease as the most common cause of death.¹ Developments in neuroimaging and other diagnostic tests have improved the ability to identify and understand the fundamental etiologies of ischemic brain lesions. In spite of these huge efforts that have been made in the search for effective treatment so far results have been quite disappointing. Stem cells in the adult brain have the potential to regenerate and repair the lesions. It is well known now that neural progenitor cells (NPC) exist in the adult brain and they have the ability to produce new neurons and glial cells during adulthood. These NPC have been found in many animal species and in the brain of adult mammals including humans.²⁻⁵ NPC are found in the subventricular zone (SVZ) lining the lateral ventricles and the subgranular zone of the hippocampal dentate gyrus.³ Several studies in experimental animal models showed that ischemic stroke induced proliferation in the SVZ and the hippocampus.⁶⁻⁷ Unfortunately it is not clear whether neurogenesis occurs in humans after stroke. Jin K Wang X *et al.*⁸ in 2006 reported that subventricular proliferation may occur in humans after stroke.

In this report the authors studied the morphologic changes and cell proliferation that occurred in the subventricular zone (SVZ) in patients who died after an acute ischemic stroke. The hypothesis of this study was to test whether NPC in the SVZ are activated after an ischemic stroke in an effort to repair the injured brain.

Study Design

The study was intended to demonstrate active cell proliferation in patients after ischemic stroke. The study included 7 elderly patients with 82 years as the mean age. For each patient clinical data was recorded such as age, sex, location of the infarct (cortical or deep), medical history, cause of death, time from stroke to death, time from death to autopsy, and weight of the brain. The mean time from the onset of stroke to death was approximately 10 days and the mean time from death to autopsy was 4 hours. The mean weight of the brain samples was 1,250

grams. Samples of post-mortem brain slices were obtained for immunohistochemistry (cellular) and microscopy (morphological) analysis. The authors selected two consecutive slices that contained both the lateral ventricle and the infarct. The equivalent slices were obtained from the contralateral side (without infarction) to serve as a control. These pair of slices were subjected to electron microscopy to study the morphological changes in the iSVZ and the cSVZ. The gap and ribbon layers containing astrocytes were found to be widened in the ipsilateral region as compared to the contralateral one indicating the occurrence of edema in these layers. Also there was an enlargement of the cytoplasmic volume of astrocytes and increase in cell density of ribbon layer.

For immunohistochemistry the central part of the SVZ was analyzed. The authors found increased Ki-67 and PCNA positive cells in the ipsilateral region of SVZ as compared to the contralateral region. They also observed a high number of mitotic cells in the ipsilateral region. Moreover, the cells that were stained for Tuj-1 a marker for immature cells and PSA-NCAM a specific marker for migrating cells were observed more prominently in the ipsilateral region. To confirm that the cells were astrocytes and not any other type of inflammatory cell like macrophage, the authors analyzed these cells with electron microscopy and found many intermediate filaments in these cells confirming these were astrocytes. Also they stained the cells with Ki-67 colabelled with GFAP together with Ki-67 colabelled with CD-68. As a result they found a higher percentage of Ki-67 colabelled with GFAP as compared to Ki-67 & CD-68 positive cells in the ipsilateral region.

Quantification of Ki-67 and PCNA positive cells was also performed using a light microscope. The authors also wanted to test whether Ki-67 positive cells represent the recent cell proliferation, they stained these cells with PCNA also. To differentiate between reactive astrogliosis and neuroblast proliferation an additional immunohistochemistry study was performed with GFAP-delta, a specific marker for neural stem cells of the adult human brain. An increased number of GFAP-delta labeling in the ribbon layer of the iSVZ was observed.

As a result of the NPC proliferation, a sixfold increase of PSA-NCAM-positive cells in the iSVZ was observed compared with the cSVZ, which indicated an increase in neuroblasts. There was a significant difference found in the percentage of PSA-NCAM positive cells observed in different layers of iSVZ and cSVZ. In gap layer the PSA-NCAM positive cells were found more pronounced as compared to the other layers indicating a significant role of this layer in migration. This report showed an increased number of migrating neuroblasts and immature neurons, indicating not only proliferation but also their migration to the site of ischemic lesion. In this report, however, migrating cells were not observed in the adjacent region of the SVZ. This could be due to the short survival time. Probably, if the patients lived longer, migrating neuroblasts would exit the SVZ and start to migrate to the ischemic area.

There are certain limitations of this study. The authors studied the brains of patients who died during the acute stage of ischemic stroke, but the time when proliferation begins or when it ends after a stroke is unclear. In addition, the brain samples were obtained from elderly patients and it is well known that the potential for neurogenesis reduces with age. If the study had included younger patients, the induction of cell proliferation might have been more distinct. The information regarding the relevant size and the exact location of the infarct (cortical vs deep infarct) is lacking. Also, it is not evident whether the intake of medication by a patient while on treatment has any effect on cell proliferation. For example, in animal models, while statins enhanced neurogenesis, corticosteroids inhibited neurogenesis. Another technical limitation in this study is that the postmortem tissue fixation was achieved by immersion. Moreover the authors did not use the extrinsic proliferating marker BrdU which could have some impact on the results.

Implications

Marti *et al.* showed that the human SVZ responds to ischemic stroke by observing a significant increase in proliferating cells in SVZ of the adult brain. They also found that these cells not only proliferate but also migrate to the site of ischemic lesion following acute ischemic stroke. They have highlighted certain limitations of this study which can be overcome in future research but current findings holds potential for the treatment of ischemic stroke.

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