Short communication

Dentate gyrus expression of nestin-immunoreactivity in patients with drug-resistant temporal lobe epilepsy and hippocampal sclerosis

L. D’Alessio, H. Konopka, E. Escobar, A. Acuña, S. Oddo, P. Solís, E. Seoane, S. Kochen

Epilepsy Center, Ramos Mejía and El Cruce Hospital, Buenos Aires, Argentina
Cell Biology and Neuroscience E de Robertis Institute, CONICET, Buenos Aires, Argentina
Moyano Hospital, Histopathology Division, Buenos Aires, Argentina

A R T I C L E  I N F O

Article history:
Received 30 December 2014
Received in revised form 3 February 2015
Accepted 12 February 2015

Keywords:
Nestin
Granule cells
Neurogenesis
Dentate gyrus neuroplasticity
Depression

A B S T R A C T

Purpose: Granule cells pathology in dentate gyrus, have received considerable attention in terms of understanding the pathophysiology of temporal lobe epilepsy with hippocampal sclerosis. The aim of this study was to determine the nestin (an intermediate filament protein expressed by newly formed cells), immunoreactivity (IR) in granular cells layers of hippocampal tissue extirpated during epilepsy surgical procedure, in patients with drug-resistant epilepsy.

Methods: Hippocampal sections of 16 patients with hippocampal sclerosis and drug-resistant temporal lobe epilepsy were processed using immunoperoxidase with antibody to nestin. Archival material from 8 normal post-mortem hippocampus, were simultaneously processed. Reactive area for nestin-IR, the total number of positive nestin cells per field (20×), and the MGV (mean gray value) was determined by computerized image analysis (ImageJ), and compared between groups. Student’s t test was used for statistical analysis.

Results: Nestin-IR cells were found in granule cells layers of both controls and patients. Larger reactive somas (p < 0.01) were found in epileptic’s sections but a significant reduction in the total number of nestin-IR cells per field and in the MGV was found in granular cells layers of patients with hippocampal sclerosis (p < 0.01).

Conclusion: Reduced expression of nestin-IR in granular cells layers of epileptic’s dentate gyrus may reflect changes in dentate gyrus neuroplasticity associated to chronic temporal epilepsy with hippocampal sclerosis. Further studies are required to determine the clinical implications on memory an emotional alterations such as depression.

© 2015 British Epilepsy Association. Published by Elsevier Ltd. All rights reserved.

1. Introduction

Hippocampal sclerosis (HS) is the most frequent lesion found in patients with drug-resistant temporal lobe epilepsy (TLE), and its resection eliminates seizures in a 60–80% of the cases [1,2]. Nevertheless, the mechanisms involved in the pathogenesis of HS are still controversial and poorly understood [3].

Despite HS is characterized by pyramidal neuronal loss and reactive gliosis, in the last years researchers have been focused on granule cell pathology dentate gyrus (DG), neurogenesis (NG) and changes in neuroplasticity (NP) with many controversial findings: while a significant increase of mitotic activity in DG was found in acute experimental models of epilepsy [4–6], a reduction of newly formed cells (NFC) have been found in chronic models of epilepsy [7–9] and in patients with drug-resistant epilepsy [10–13]. Furthermore, a reduction in DG NG and NP have been associated to cognitive deficits and emotional disturbances (depression), frequently observed among TLE patients [14,15].
In a previous study, we found a decreased in doublecortin (DCX)\(^7\) immunoreactivity (a marker used to determine NFC in the late stages of NG) in adult patients with TLE and HS\(^{16}\). The aim of the present study was to determine nestin immunoreactivity (nestin-IR), an intermediate filament protein expressed in early stages of differentiation of NFC, in granule cells layers of DG obtained from patients with HS and chronic TLE, who underwent epilepsy surgery.

2. Methods

2.1. Patients and samples

Hippocampal sections from patients with TLE and HS who underwent epilepsy surgery (anterior temporal lobectomy) were included in this study. All patients underwent thorough clinical, electrophysiological (video-EEG), imaging evaluation (magnetic resonance imaging – MRI), neuropsychological and psychiatric assessment prior to surgery\(^{17}\). Pharmacoresistance was defined as failure to achieve sustained seizure absence (no type of seizures for a period of 12 months, or prolongation of three times the preintervention inter-seizure interval, which ever longer), with at least two trials of well tolerated, appropriately chosen, and adequate schedules AED (irrespective of being administered as monotherapy or in combination), to achieve sustained seizure absence\(^{18}\).

Archival material obtained from post-mortem hippocampus matched by gender and age, free from neurological injury, drug and/or alcohol dependency were simultaneously processed as controls.

This study was conducted with the approval of the Ethics Committee of Ramos Mejía Hospital of Buenos Aires Argentina, in accordance with the Ethical Standards laid down in the 1964 Declaration of Helsinki, and all the subjects submitted informed consent.

2.2. Hippocampal sclerosis diagnoses

2.2.1. Magnetic resonance image (MRI)

Magnetic resonance image (MRI)\(^5\) protocol used was sagittal T1-weighted, inversion-recovery, fluid-attenuated inversion recovery (FLAIR) T1 FFE 3D acquisition, perpendicular to the long axis of the hippocampus and T2-weighted axial, parallel to the long axis of the hippocampus. Diagnostic Criteria for HS by MRI was atrophy, hypointense in T1W and IR, hyperintense in T2W and Flair, and alteration of the internal structure of the hippocampus.

2.2.2. Tissue processing and histopathology diagnosis

Hippocampal samples were studied by a neuropathologist to confirm HS diagnoses and were classified according to Blümke et al.\(^{2}\) criteria. The surgical piece was fixed in formalin for 5 days. After that, tissue blocks (thickness: 5 mm) were made following coronal planes and were embedded in paraffin. Sections were cut at 7 mm with a microtome, stretched in water at ambient temperature and mounted on slides, deparaffined in xylene, hydrated and stained with hematoxylin-eosin, luxol fast blue and thionin stain. Archival material obtained from normal post-mortem hippocampus matched by gender and age, and free from neurological injury, drug and/or alcohol dependency were simultaneously processed as controls.

2.2.3. Immunohistochemistry

After deparaffinizing sections were treated according to the following procedure: a 15-min wash in distilled water, then an incubation in a microwave oven twice for 5 min in a citric acid solution (0.1 mol/L citric acid monohydrate and 0.1 mol/L trisodium citrate dihydrate), pH 6.0; after that a twofold 5-min wash in phosphate-buffered saline (PBS), the sections were incubated for 30 min in 0.5% (w/v) hydrogen peroxide in ethanol to quench endogenous peroxidases. Afterwards, they were incubated overnight in a humid chamber with mouse monoclonal anti nestin (EMD Millipore) diluted 1:200 in PBS Triton X-100 and 0.1% (w/v) sodium azide. The complex was detected using supersensitive multilink-HRP/DAB kit from Bio-Genex (QD000-5L) following the vendor’s procedure. After dehydration the sections were mounted with permount medium and coverslipped.

Controls omitting the primary and the secondary antibody were determined.

2.2.4. Image analysis

Immunocytothermometric evaluation and quantification of granule cells layers of DG expressing nestin (nestin-IR)\(^9\) was determined by computerized image analysis. The images were acquired by a SONY. Power Had 3CCD color video camera system from a Zeiss Axioskop microscope. Images were digitalized with a resolution of 768–494 pixels and were analyzed using ImageJ analysis program. All images were captured under identical lighting and magnification conditions. Ten fields per section were evaluated in cases and controls. The total number of positive nestin-IR cells by field (20×), the mean gray value (MGV) and the reactive area (pixel\(^2\)) were measured along the granular layers. After shading correction, an automatic discrimination procedure was performed and the MGV of specific labeling and the background was measured. The specific MGV was defined as the difference between the background MGV and the MGV of the discriminated profiles, indicating a measure of staining intensity. Student t test was used for statistical analysis.

2.2.5. Statistical analysis

Student's t test was calculated, \(p < 0.01\) was considered significant. SPSS for Windows was used to perform statistical analysis.

3. Results

In this study hippocampal samples obtained from 16 p.\(^10\) 7 women (44%) and 11 men (65%), mean age 38 ± 8 years, with TLE and eight post-mortem controls, age 39 ± 17.4 without pathology, matched by age and sex (\(p > 0.05\)) were included. Demographic, clinical and histopathological variables are resumed in Table 1.

In both control and epileptic sections, positive nestin-IR cells were found among pyramidal layers, subpial zones, and in granular cells of DG layers.

Along DG layers nestin-IR granular-like cells were localized preferentially in somas in both cases and controls. Positive nestin-IR cells in epileptic’s granular cell layers had larger reactive somas. Many of these cells have ectopic localization, and were found into the molecular layers. On the contrary a reduction in the total number of nestin-IR cells per field (20×) and a lower MGV (indicating a reduce staining intensity of nestin-IR) was found in epileptic’s granular cell layers (\(p < 0.01\)) (Figs. 1–2).

\(^7\) DCX: doublecortin.
\(^5\) MRI: magnetic resonance image.
\(^9\) NC: nestin positive cells.
\(^10\) p.: patients.
Furthermore, synaptic activity identifies a process with considerable functional integration [19–21]. Nevertheless, any epileptic insults, such as an antiepileptic drug, carbamazepine, VPA: valproate, TPM: topiramate, OCX: oxcarbazepine, LVT: levetiracetam, Engel class: Engel classification of postsurgical seizure outcome [23].

4. Discussion

Despite nestin expression in the adult brain is also detected in pyramidal cells in the adult neocortex, and in reactive astrocytes following injury, nestin-IR in granule cells of DG may directly identifies NFC and marks the NFC which will differentiate into neurons [19–21]. Granule cells pathology DG NG and DG NP have received considerable attention in terms of understanding the pathophysiology of TLE. The process of adult NG is a multi-step process (proliferation, differentiation, migration, targeting, and synaptic integration), that ends with the formation of a postmitotic functionally integrated new neuron [19]. It has been proposed, that chronic and recurrent epileptic discharges, as occurs in adults patients with a long history of resistant epilepsy affects the granular layers of DG, and modifies the pattern of NFC process in different levels affecting synaptic integration and NP [9].

Nevertheless, there are controversial findings between acute and chronic models of epilepsy. A significant increase of mitotic activity in DG was found in acute experimental models of epilepsy [4–6]. At early time points after acute epileptic insult, DG dramatic increases the production of new neurons (proliferation) and after that, aberrant migration infiltrate into the dentate hilus and molecular layer and integrate abnormally into the CA3 network [5]. On the contrary to these observations, chronic models of experimental epilepsy found that initial seizure-induced neurogenesis returns to the baseline by about 2 months in rats [22] and reaches substantially below the baseline level by 5 months [8]. Furthermore, repeated seizures (more than 10) reduce NG in DG [10] and the number of newly born cells that migrate abnormally into the dentate hilus (i.e., ectopic granule cells) are significantly reduced in older age [23].

Coinciding with these observations, a reduction of NFC were found in patients with chronic TLE, using protein markers with indirectly detect human DG NG (direct techniques that mark new DNA synthesis are not ethical to use in humans) [19]. A reduction of PSA-NCAM (polysialylated-neural cell adhesion molecule) was found in DG of children with resistant epilepsy [11] and in TLE patients with severe neuronal loss [24], the absence of Ki-67 immunopositive nuclei (a proliferative marker of early stages of NG) and a reduction of minichromosome maintenance protein 2 (mcm2) (a proliferative marker) was found in DG of ELT patients [13] and a reduction of doublecortin (DCX) (late differentiation events of NG) was described in chronic TLE patients [8,9,13,16].

There are still controversies since other authors found no differences in the expression human NG markers [25], or as well found a higher number of mcm2–positive cells, Ki-67 and nestin, suggesting higher NFC determined in earlier stages of NG [26–28]. Nevertheless, to explain these controversial findings it has been proposed that chronic epilepsy acting through time, affects the survival of NFC with a decline in the neuronal differentiation process [29].

In this exploratory study, we found a reduce number of nestin-IR cells with lower levels of nestin staining intensity in granular cells layers of epileptic’s dentate gyrus. These findings may reflect a reduces number of NFC and changes in DG NP associated to chronic TLE and HS. A reduced NP in the DG may have clinical consequences, and may be related to memory deficits and depression, which are frequently associated to chronic and recurrent seizures in drug-resistant TLE [2,15]. NFC are reduced in experimental models of depression and antidepressants enhances NG [14,15]. Furthermore a depletion of granule cells, has been related to memory impairment in patients with TLE [2,29]. In the present study, memory deficits were presented in nearly 70% and psychiatric comorbidities in almost half of patients. Some limitations of this preliminary study must be mentioned. The aim of this exploratory study was to localize and quantify nestin-IR in granular cells of DG of patients with HS, and to compare them with normal tissue. In this work, we cannot rule out if the reduced number of nestin-IR cells are based on an overall reduced number of granular cells, described in HS specially in grade 2 of dentate gyrus dispersion [2, or not. Nevertheless a
Conflict of interest

The authors do not declare any conflict of interest.

Acknowledgments

We thank all study participants and collaborators.

References

Seizures in the hippocampus and the dentate gyrus during temporal lobe epilepsy may be due to aberrant neurogenesis, characterized by the presence of nestin-immunoreactive cells in the dentate gyrus. These cells are likely formed by retrovirus-mediated stem cell proliferation in the dentate gyrus of patients with mesial temporal lobe epilepsy. The number of nestin-immunoreactive cells in the dentate gyrus is increased in pediatric patients with early-onset temporal lobe epilepsy compared to healthy controls. This increase is accompanied by a decrease in the number of immature granule cells in the dentate gyrus, suggesting a defect in granule cell differentiation. The role of aberrant neurogenesis in the pathogenesis of temporal lobe epilepsy remains to be elucidated, but it is likely that these mechanisms contribute to the heterogeneous clinical presentation of this disorder.

References:


