Neuroscience Letters 566 (2014) 188–193

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Increased population of oligodendroglia-like cells in pediatric intractable epilepsy

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HIGHLIGHTS

- Oligodendroglia-like cells (OLCs) were found in pediatric intractable focal epilepsy.
- The population of OLCs in pediatric epileptic brain are significantly increased.
- The largest population increase of OLCs is in the white matter.
- OLCs may contribute to the extensive epileptic network in children.

ARTICLE INFO

Article history:
Received 12 November 2013
Received in revised form 27 February 2014
Accepted 1 March 2014

Keywords:
Intractable focal epilepsy
Pediatric epilepsy surgery
Oligodendroglia-like cells
Oligodendroglia
Olig2
Histopathology

ABSTRACT

Pediatric focal epilepsies often involve more extratemporal regions than adult epilepsies. This study aims to investigate the population of oligodendroglia-like cells (OLCs) in the pediatric focal epilepsy patients requiring surgery. We hypothesize that OLCs are one of the factors that extend the pediatric epileptic network in intractable epilepsy. Thirty (18 female) patients (1.8–16.9 years old with a mean of 9.7 years), who underwent resective surgery for the intractable epilepsy from 2010 to 2012 were retrospectively studied. Seizure types consisted of epileptic spasms in nine patients, partial seizures in 17 patients and partial seizure with secondary generalization in four patients. Eight autopsy cases without neurological disease served as controls. The neuropathology examination utilized the H&E/LFB stain and immunohistochemical staining for NeuN, GFAP and Olig2 as a marker of OLCs. OLCs were counted in three sites: (a) gray matter, (b) junction of gray/white matter, and (c) white matter. We also examined the correlation between the density of OLC among the three sites and the clinical features. Fifteen (50%) patients underwent multiple lobe resections, consisting of both temporal and extratemporal lobe resections in 12 patients and extratemporal lobe resections in 3 patients. The other 15 (50%) patients underwent single lobe resection including 3 (10%) patients with temporal lobectomy sparing hippocampus. Pathological diagnosis of epilepsy patients was as follows: 14 (47%) patients = focal cortical dysplasia (type I, 4; II, 9; III, 1); 6 (20%) = oligodendrogliosis; 6 (20%) = astrocytic gliosis; 2 (7%) = hyaline protoplasmic astrocytopathy and 2 (7%) = tuberous sclerosis complex. The numbers of OLCs at all three sites in epilepsy group were significantly higher than those of control group ($p < 0.001$). In the epilepsy group, there was a significant difference among the number of OLCs at gray matter, junction of gray and white matter, and white matter ($p < 0.001$). The number of OLCs significantly increased from gray matter and junction of gray/white matter to white matter. In the control group, there was no difference among the number of OLCs at three sites. There was no significant difference in the numbers of OLCs between focal cortical dysplasia types I and II. The significantly increased OLCs, especially in the white matter may contribute to the extensive epileptic network in children with intractable focal epilepsy.

1. Introduction

It is well known that neuronal abnormalities are associated with epilepsy. Glial cells and their pathologies also have an influence on epileptogenesis. Reactive astrocytes are observed in epileptic brain [4]. Astrocytic cytoplasmic inclusions were observed in the brains

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http://dx.doi.org/10.1016/j.neulet.2014.03.002
0304-3940/

of patients with intractable epilepsy [10–12]. The white matter has an increased population of cells, termed oligodendroglia-like cells (OLCs), which have a round nuclei and a scant amount of cytoplasm [26]. Clusters of oligodendroglia or OLCs are found in epilepsy surgery specimens [18].

Oligodendrocytes are the end product of a cell lineage which has to undergo a complex and precisely timed program of proliferation, migration, differentiation, and myelination to finally produce the insulating sheath of axons [3]. Oligodendrocytes can also provide trophic supports for neurons by producing glial cell line-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), and insulin-like growth factor-1(IGF-1) [7,8,25].

Pediatric focal epilepsies often involve more extratemporal regions than adult epilepsies [5,19]. Multiple lobar resection are required in children with epileptic spasms [13]. Inage and co-workers noted clusters of oligodendroglia were seen in a subset of patients with epileptic spasms and partial seizures. They speculated the abnormalities of oligodendroglia might spread the epileptogenic zone. OLCs were increased in the white matter of adolescence and adult patients with mesial temporal sclerosis [6]. Prominent oligodendroglial response has been found in the white matter of adult patients with temporal lobe epilepsy [23]. Komori et al. described that the histological appearance of the perivascular small round cells in this lesion was reminiscent of that of neurocytes, oligodendroglia or OLCs in two pediatric patients [16].

The epileptic network has been considered as functionally and anatomically abnormal neuronal networks to propagate seizure and results in extending epilepsy [22]. We hypothesize that OLCs is one of the factors to extend the pediatric epileptic network in the intractable epilepsy. This study aims to investigate the population dynamics of OLCs in the pediatric focal epilepsy patients requiring resective surgery.

2. Patients and methods

2.1. Subjects

We retrospectively collected 30 (18 female) patients who underwent the resective surgery for the intractable focal epilepsy, following the intracranial video EEG from 2010 to 2012 at the Hospital for Sick Children, Toronto, Canada. As controls, we collected eight autopsy cases without neurological disease from the first two decades of life. We selected control cases with a post-mortem interval within 48 h for Olig2 immunostaining [9]. We reviewed age, gender, seizure type, resection area and diagnosis from medical records. This study was approved by Research Ethics Board, The Hospital for Sick Children.

2.2. Tissue preparation and immunohistochemistry

The surgical specimens were fixed in 10% neutral buffered formalin, serially sectioned in a plane perpendicular to the cortical surface and sampled for light microscopy. Paraffin embedded tissue, cut at 5 microns, were used for staining (Hematoxylin and Eosin/Luxol Fast Blue stain) and immunohistochemistry. For immunohistochemical studies, Anti-glial fibrillary acidic protein (GFAP, diluted 1:2500; Cat# Z0334, DAKO, Glostrup, Denmark), anti-neuronal nuclear antigen (NeuN, diluted 1:50, Cat# MAB377, Millipore, Temeuza, CA, USA) and anti-Olig2 (Olig2, diluted 1:200; Cat# AB9610, Millipore, Temeuza, CA, USA) were utilized. To assess the specificity of primary antibodies, we used the mouse monoclonal antibody (diluted 1:200; Cat# 760-2014, VENTANA, AZ, USA) as a negative control. Anti-Olig2 antibody was utilized as a marker of OLCs. Olig2 is a transcription factor that controls development and differentiation of the oligodendrocyte. Olig2 is strongly expressed in oligodendrocyte precursor cells/progenitors and weakly expressed in mature oligodendrocyte [3,17].

2.3. Count of the OLCs

The neuropathology reviewers, who were blind to the case information, analyzed the OLCs in the specimens. The slides for counting were chosen from those with the most prominent expressions of Olig2 in the white matter. Examining the pathology of subcortical lesion is needed for elucidating why epileptic network have been expanded in pediatric intractable epilepsy. OLCs were counted in three sites: (a) gray matter, (b) junction of gray and white matter, and (c) white matter with a light microscope (ECLIPSE E400, Nikon, Japan; magnification 200×). We counted the number of OLCs in the white matter, approximately 300–500 μm away from the gray/white matter junction. The area of counting OLCs was determined as 1 mm^2. The number of OLCs were visually counted using ImageJ 1.48a software (NIH, Bethesda, MD, USA). For each site, the number of OLCs was calculated by taking the mean of the total number observed in three different fields of a slide in each case. We compared the number of OLCs in epilepsy surgery cases to those of controls using the Student's *t*-test and SSPS16.0J software (SPSS Inc., Chicago, IL, USA).

3. Results

Table 1 outlines the age at surgery, gender, seizure types, surgical resection areas, and the number of OLCs at each of the three sites.

3.1. Subjects profile

Of the 30 patients with intractable epilepsy, the age of epilepsy surgery ranged from 1.8 to 16.9 years old with a mean of 9.7 years. The age of seizure onset ranged from 0.3 to 13.5 years old with a mean of 4.8 years. The duration of seizures ranged from 0.4 to 12.5 years with a mean of 4.9 years. Seizure types consisted of epileptic spasms (ES) in nine patients, partial seizures in 17 patients and partial seizure with secondary generalization in four patients.

Of the eight control cases, the age of autopsy ranged from 2–16 years old with a mean of 9.4 years (Table 1). The brain tissue was collected from frontal lobe in six cases, parietal and occipital lobe in one each. The post-mortem interval ranged between 13 and 48 h with a median of 24.5 h.

3.2. Resection areas in epilepsy patients

Three (10%) patients underwent temporal lobe resection, which spared hippocampus. Fifteen (50%) patients underwent extratemporal lobe resection. The remaining 12 patients underwent both temporal and extratemporal lobe resection.

Fifteen (50%) patients underwent multiple lobe resections, consisting of both temporal and extratemporal lobe resection in 12 patients and extratemporal lobe resection in 3 patients. Eight (89%) of nine patients with ES had multiple lobe resection. Fourteen (67%) of twenty-one patients with partial seizures and partial seizure with secondary generalization had a single lobe resection. In comparison to patients with partial seizures and partial seizure with secondary generalization, patients with ES tended to need multiple lobe resections (Fisher's exact test; $p < 0.05$, odds ratio = 16.0).

3.3. Neuropathological diagnosis

In the neuropathological examination, the diagnosis was as follows: 14 (47%) patients were focal cortical dysplasia (type I, 4; II,