Journal of Neuroimmunology 254 (2013) 125–130

Contents lists available at SciVerse ScienceDirect

Journal of Neuroimmunology

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

Resection of the epileptogenic lesion abolishes seizures and reduces inflammatory cytokines of patients with temporal lobe epilepsy

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ARTICLE INFO

Article history:
Received 21 September 2011
Received in revised form 28 March 2012
Accepted 9 August 2012

Keywords:
Lobectomy
Mesial temporal lobe epilepsy
Seizure
Cytokine
Neuroinflammation

ABSTRACT

Persistent neuroinflammation is implicated in the pathogenesis of seizures and neuronal degeneration of temporal lobe epilepsy (TLE). Circulating level of inflammatory cytokines was determined during inter-ictal period of 25 non-operated and 10 patients (OP) submitted to anterior temporal lobectomy. OP patients showed marked reduction of IL-1β, TNFα, MIP-1α, but not IL-6 and TGF-β1. Paired analysis done before and after lobectomy showed reduction of inflammatory cytokines but increased TGF-β1 levels, and lack of seizures for more than 6 months. Maintenance of high TGF-β1 and IL-6 cytokines in both groups suggests a role in down-regulation of neuroinflammation and promotion of brain tissue remodeling for neuronal reorganization.

1. Introduction

Mesial temporal lobe epilepsy with hippocampal sclerosis (TLE-HS) is a frequent condition within the pharmacoresistant group of epilepsies classified as a syndrome with clinical, electroencephalographic, genetic and immunological features (Berg, 2009; Vezzani et al., 2011). Indication that TLE-HS is a progressive disease was established after evidence that initial precipitant incidents (IPI) in childhood was followed by a latent period until onset of spontaneous seizures at later age (Wieser, 2004). The release of inflammatory cytokines in mesial structures may activate mechanisms associated with prolonged epileptic seizure and neuronal damage (Balosso et al., 2009), and also neurogenesis, neuroplasticity and synaptic reorganization (Jankowsky and Patterson, 2001; Vezzani et al., 2011).

TNF-α proinflammatory cytokine is expressed at low levels in healthy brain but is rapidly upregulated in glia, neurons and endothelial cells during seizures (Fabene et al., 2010). Transient transcription of IL-1β, transforming growth factor beta (TGF-β1) and overexpression of TNFα and IL-6 after induced *status epilepticus* in experimental models (Minami et al., 1991; De Simoni et al., 2000) indicate that activation of cytokine signaling cascade influences development of recurrent seizures and brain tissue remodeling (Lehtimaki et al., 2007; Aronica and Crino, 2011; Lehtimaki et al., 2011). Additionally, altered cytokine plasma levels have been associated with the frequency and severity of temporal seizures, and also with focal and generalized epilepsy during interictal period (Lehtimaki et al., 2007; Mlodzikowska-Albrecht et al., 2007; Alapirtti et al., 2009; Nowak et al., 2011). Such evidences support the hypothesis that dysregulation and/or excessive cytokine production could induce seizures and lead to neuronal degeneration in susceptible subjects. This work aimed to determine the circulating level of cytokines (TNFα; IL-1β; IL-6, sTNFR1, TGF-β1 and CCL3/MIP-1α) that influence inflammatory responses predisposing to seizures and/or tissue remodeling in adult central nervous system. In addition we assessed whether ablation of epileptogenic lesion of TLE patients with hippocampal sclerosis and active pharmacoresistant epilepsy would improve clinical condition and reduce seizures.

2. Materials and methods

2.1. Patients

This prospective study carried out from October 2009 to December 2010 in the Clementino Fraga Filho University Hospital included after appropriate informed consent, 35 patients with chronic TLE (19 males and 16 females) attending Neurology and Neurosurgery services at a tertiary care university medical hospital. The Hospital Medical Research Ethics Committee and the Brazilian Ministry of Health (CONEP 073-2007) approved this study that complies with the principles laid down in the Declaration of Helsinki. Diagnosis and classification of mesial temporal

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0165-5728
http://dx.doi.org/10.1016/j.jneuroim.2012.08.004

lobe epilepsy with hippocampal sclerosis was based upon previous criteria (Wieser, 2004; Engel, 2006).

Thirty-five patients with medically intractable TLE and hippocampal sclerosis were under investigation for surgery. Among them a group of 10 patients with high (6 to 14 seizures by month) seizure frequency underwent anterior temporal lobe resection and amygdalohippocampectomy (OP). Patients investigated for the history of initial precipitant events (IPI) reported febrile seizure as the most frequent event. At the time of blood collection patients were free from clinical signs of infection and seizure for at least a week, considering that cytokine production is upregulated within 24 h from seizure occurrence (Lehtimaki et al., 2007). Blood samples from OP patients were also collected 48 h before and 8 weeks after surgery. Reference range of plasma cytokines was obtained from 42 (28 males, 66.6%) healthy subjects from the blood transfusion HEMONIT Centre at Fluminense Federal University with age range 18 to 61 years (36.8 ± 9.56 years). Eligibility of control group was assessed by detailed questionnaire, and was only included subjects with no familial history of epilepsy, infectious disease or under use of anti-inflammatory medication.

TLE patients were submitted to a comprehensive investigation and neurological examination which included at least a 4-day video-electroencephalogram (video-EEG) recording, magnetic resonance imaging (MRI), neuropsychological evaluation, and ictal single photon emission computed tomography (SPECT). Latency period was defined as the interval between initial precipitating event (IPI) and the first reported spontaneous epileptic seizure, and silent period as the lag-time between the first seizure and initial clinical evidence of pharmacoresistance (Berg, 2009).

Criteria of eligibility included clinical diagnosis of intractable TLE based on seizure semiology (Luders et al., 1998), ictal video-EEG and 1.5 T MRI of the brain with characteristic image alteration. All patients were good candidates to surgery because they presented unilateral ictal register concordant with unilateral sclerotic hippocampus. Exclusion criteria included occurrence of seizures within the last 24 h before inclusion; concomitant neoplasm; concomitant infectious or inflammatory disease; acute severe cerebrovascular disorder; use of immunomodulatory drug within the last 6 months and/or pregnancy. Also, it was not included patients with genetic syndromes, genetic disorders, cortical dysgenesias, acquired encephalopaties, or autism.

2.2. Surgical procedure

Anterior temporal lobe resection and amygdalohippocampectomy were performed in 10 patients by a single neurosurgeon that made consistent measurements of resection parameters during surgery. After surgery, patients continued to use the same antiepileptical drugs (carbamazepine, phenytoin, oxcarbazepine, valproic acid). No neurological and/or cognitive deficits were observed following surgery, and postoperative MRI revealed no further complications.

2.3. Blood collection and cytokine assays

5 mL blood samples collected at the same time-matched period (8 a.m. to 10 a.m.) to minimize influence of circadian rhythm in the cytokine release were stored at −80 °C and thawed once just prior cytokine analysis. TNFα, sTNFR1, IL-6, CCL3/MIP-1α and TGF-β1 (R&D-Systems, Minneapolis, MN) and IL-1β (eBioscience, San Diego, CA) were quantified in triplicate by commercial enzyme-linked immunosorbent assays (ELISA) according to the manufacturer's instructions. In 10 patients blood samples were also collected 48 h before and 8 weeks after surgery for comparison of cytokine levels.

2.4. Statistical analysis

Statistical analysis was carried out using the GraphPad Prism software (GraphPad Software Inc., San Diego, CA) with tests analyzing differences between groups (Mann–Whitney *U*-test and Wilcoxon signed rank test), differences between OP and nOP were tested with unpaired *t*-test with Welch's correction, one-way ANOVA test with Dunn's Multiple Comparison test for multiple comparisons and Mann Whitney paired test for analysis before and after surgery. The significance level was set to $p<0.05$.

3. Results

All patients (n = 35) with temporal lobe epilepsy under investigation for surgery had a characteristic pattern of selective and extensive hippocampal atrophy (Table 1) and were under treatment with anticonvulsant polytherapy medication. In order to accurately define the epileptogenic focus it was performed a careful and detailed clinical semiology including a 4-day video-EEG recording, a 1.5 T MRI, and also single photon emission tomography (SPECT) which indicated that patients had TLE characterized as unilateral ictal mesial temporal lobe epilepsy. During the period of the study, 10 patients (OP) with high (6 to 14) seizure frequency that had been submitted to surgery were seizure-free for more than 6 months whereas 25 patients (nOP) with similar epidemiology but still under clinical investigation for surgery maintained high seizure frequency with 6.5 (range 2 to 12) mean epileptic seizure per month. Such result confirms that sclerotic hippocampus is the main source of electrical events that cause spontaneous epileptic seizures (Spencer, 1998), and emphasize the importance of pre-operative MRI and SPECT for critical selection of patients regarding surgical therapy (Jeha et al., 2006; Vale et al., 2012). Moreover clinical outcome based on Engel classification (Engel, 2006; Vale et al., 2012) indicated that 85% of patients were classified as Engel Class-Ia and 15% Engel Class-Ib as favorable parameter of seizure-freedom outcome for more than 6 months following anterior temporal lobe resection and amygdalohippocampectomy.

In order to establish a possible association of neuroinflammation with this form of epilepsy, we compared TNFα, IL-1β, IL-6 and CCL3/MIP-1α plasma levels between TLE patients (n = 35) and healthy subjects (n = 42). TLE patients showed (Fig. 1) a five-fold increase of

Table 1
Demographic characteristics of patients with intractable TLE.
All patients were under treatment with two or more anticonvulsant medications of carbamazepine, phenytoin, oxcarbazepine, and valproic acid. Normal MRI means absence of structural abnormality of brain.

Characteristics	Operated[a]	Non operated
	N = 10	N = 25
Male	6 (60%)	13 (52%)
Female	4 (40%)	12 (48%)
Mean age years (range)[a]	39.8 (27 to 59)	39.7 (16 to 51)
Mean age of seizures at onset	13 (1 to 35)	12 (1 to 44)
Onset of seizures (*n*)		
≤18 years	3 (30%)	11 (44%)
≥18 years	7 (70%)	14 (56%)
Mean time of disease duration	25.5 (6 to 41)	28 (6 to 50)
Course of epilepsy (*n*)		
<10 years	2 (4 to 8)	3 (2 to 9)
>10 years	8 (10 to 31)	22 (11 to 40)
Frequency of seizures per month (*n*)	0	6.5 (2 to 12)
3–4	0	4 (16%)
5–8	4 (40%)	19 (76%)
>8	6 (60%)	2 (8%)
History of IPI	Present 4 (40%)	Present 7 (28%)
Family history of epilepsy (*n*)		
Yes	2 (20%)	8 (32%)
No	8 (80%)	17 (68%)
Abnormal MRI (*n*)	4 (40%)	7 (28%)
TLE (left)	8 (80%)	19 (76%)
Latency period (median in years)	22.5 (6 to 35)	10 (4 to 14)
Silent period—(median in years)	9.87 (0 to 25)	9.0 (0 to 33)

[a] Before surgery.