Brain expression of inflammatory mediators in Mesial Temporal Lobe Epilepsy patients

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ABSTRACT

Neuroinflammation may be central in epileptogenesis. In this study we analysed inflammatory reaction markers in brain tissue of Mesial Temporal Lobe Epilepsy with Hippocampal Sclerosis (MTLE-HS) patients. TLR4, IL-1β and IL-10 gene expression as well as the presence of activated HLA-DR + microglia was evaluated in 23 patients and 10 cadaveric controls. Inflammation characterized by the presence of HLA-DR[+] microglia and TLR4, IL-1β overexpression was evident in hippocampus and anterior temporal cortex of MTLE-HS patients. Anti-inflammatory IL-10 was also overexpressed in MTLE-HS patients. Our results show that hippocampal neuroinflammation extends beyond lesional limits, as far as the anterior temporal cortex.

1. Introduction

Active inflammation has been documented not only in traditionally assumed inflammatory epilepsies but also in patients with pharmacoresistant epilepsy of diverse causes (Vezzani et al., 2011a; Vezzani and Ruegg, 2011). MTLE-HS is the more frequent partial epilepsy in adulthood. It is usually refractory with over 80% patients presenting a poor response to conventional anti-seizure drugs (ASDs). Refractory patients are often subjected to surgical resection of the hippocampus and amygdala in order to control seizures. This is one of the most successful epilepsy surgeries. Nevertheless, it is reported a seizure recurrence of 38% at 18 years of follow-up after surgery (Hemb et al., 2013). For MTLE-HS patients the efficient resolution of seizures is still an unmet clinical need. Understanding the epileptogenic process is fundamental for the development of new ASDs, but the mechanisms leading to MTLE-HS remain largely unknown.

Retrospective studies show that MTLE-HS patients often have a history of initial precipitating injury such as febrile seizures (FS),

central nervous system infection and head trauma or hypoxia peripartum (Fisher et al., 1998). Among these factors FS is the most common with up to 80% of MTLE-HS patients reporting a history of complex FS (Fisher et al., 1998; Hesdorffer et al., 2016). It has been hypothesized that after the initial insult there is an abnormal cascade of damage repair, with the maintenance of chronic inflammation, leading to atrophy and hippocampal sclerosis (Fisher et al., 1998; Gallentine et al., 2017; Lewis et al., 2014). In fact, imaging studies have shown that prolonged and lateralized FS can produce acute hippocampal injury with oedema that resolves within 5 days (Scott et al., 2003). The follow-up of these children showed changes in hippocampal symmetry consistent with injury and neuronal loss (Scott et al., 2003; Shinnar et al., 2012). So, it is believed that FS initiate the abnormal network reorganization that will lead to the development of an epileptogenic structure. Alternatively, the asymmetry could represent a return (post-acute oedema) to a pre-existing hippocampal abnormality (Fernandez et al., 1998; Lewis et al., 2014).

In normal physiological conditions pro-inflammatory cytokines,

such as TNF-α, IL-1, and IL-6, and their receptors are constitutively expressed at low levels in different brain regions by astrocytes, microglia cells, neurons and endothelial cells (Vezzani and Viviani, 2015). These proteins are claimed to be important in neuronal development, controlling neurite outgrowth, neurogenesis and cell survival (Vezzani and Viviani, 2015). These pro-inflammatory cytokines can also modulate voltage-gated and receptor-coupled ionic channels (Viviani et al., 2007) as well as neurotransmitter's receptors (Balosso et al., 2009; Roseti et al., 2015; Stellwagen et al., 2005) controlling synaptic pruning, transmission and plasticity in the adult brain (Marin and Kipnis, 2013). In fact, IL-1β and IL-6 seem to have a general inhibitory action on CNS voltage-gated channels inhibiting Ca^{2+}, Na^{+} and K^{+} currents (for review see (Vezzani and Viviani, 2015)). In ligand-gated ion channels, IL-1β is observed to be mainly excitatory increasing NMDA-mediated Ca^{2+} influx (Viviani et al., 2003). Additionally, IL-1β can promote excitability through the downmodulation of the astrocytic glutamate transporter (GLT-1) (Hu et al., 2000) while promoting the release of excitatory neurotransmitters such as Glutamate or ATP (Devinsky et al., 2013). In fact, neuroinflammation appears to be an important component in epileptogenesis, reflecting complex cross-talks between microglia, astrocytes and neurons (Aronica et al., 2012; Devinsky et al., 2013).

Cytokines can also influence the strength of synaptic transmission as they can modulate NMDA, AMPA and GABA(A) receptor expression and their sub-unit composition. In accordance, several studies demonstrated that a fine-tuned cytokine production is necessary for learning and cognition and that a dysregulation may lead to excitotoxicity (McAfoose and Baune, 2009; Yirmiya and Goshen, 2011).

Seizure-induced cell death leads to the release of endogenous molecules (DAMPs) such as HMGB1 that are recognized by TLRs expressed in glial cells and neurons (Bianchi, 2007; Mazarati et al., 2011). The engagement of TLRs leads to the activation of innate immunity with the production of pro-inflammatory mediators. In fact, it has been demonstrated in animal models that several inflammatory cytokines (such as IL-1β, TNFα and Il-6) as well as TLRs (for example TLR4 and TLR9) are rapidly induced by seizures in activated astrocytes and microglia (De Simoni et al., 2000; Maroso et al., 2010; Ravizza et al., 2005; Tan et al., 2015; Vezzani et al., 1999). Also, microarray studies in rodent models of TLE showed that inflammation is one of the most upregulated biological processes during epileptogenesis (Gorter et al., 2006). Inflammatory molecules contribute to decrease seizure threshold by direct effects on neuronal excitability (Vezzani and Baram, 2007) and may also activate transcription of genes involved in neurogenesis, cell death, and synaptic plasticity (Vezzani et al., 2008b; Widera et al., 2006). In this way, inflammatory molecules can also participate in glial scar formation contributing to seizure-related hippocampal changes, such as neuronal loss, reactive gliosis and mossy fiber sprouting.

These experimental results are corroborated by studies in human brain tissue from drug-resistant epilepsies. In these patients activation of hippocampal microglia with concomitant over-expression of HLA-DR (Beach et al., 1995) and upregulation of diverse inflammatory mediators, such as complement proteins or pro-inflammatory cytokines, has been evidenced (Aronica et al., 2007; Choi et al., 2009; Crespel et al., 2002; Jamali et al., 2006; Kan et al., 2012; Omran et al., 2012; Ravizza et al., 2008). Upregulation of several inflammatory players has also been observed in cerebrospinal fluid and serum of epileptic patients (de Vries et al., 2016). Moreover, it is known that some conventional ASDs have an anti-inflammatory action (Matoth et al., 2000) and that administration of anti-inflammatory drugs can also have anti-convulsant effects with reduction of seizures (Hancock et al., 2013; Radu et al., 2017; Vezzani, 2015). On the other hand, anti-inflammatory cytokines such as IL-10 may protect against seizures (Ishizaki et al., 2009).

Studies using surgically-removed anterior cortical region are scarce. This region is thought to contribute to seizure propagation in MTLE-HS patients (Bartolomei et al., 2008). Thus, the aim of this study was to characterize the expression of inflammatory mediators namely, IL-1β,

Table 1
Clinical and demographic data from surgery MTLE-HS patients.

Clinical/demographic data	MTLE-HS (n total = 23)
F/M	13/10
Age at surgery ± SD, years (range)	39.6 ± 9.8 (24–60)
Age of onset ± SD, years (range)	10.3 ± 6.8 (1–28)
Disease mean duration ± SD, years (range)	29.3 ± 9.0 (10–49)
Post-surgery time ± SD, years (range)	6.9 ± 1.3 (5–10)
Hippocampal Sclerosis (Left/Right)	15/8
Febrile seizures antecedents (Yes/No)	15/8
Engel classification (I/II/III/IV)	16/2/4/1

TLR4, and IL-10, both in the hippocampus and in the anterior temporal cortex.

2. Material and methods

2.1. Population

Resected fresh human tissue obtained from 23 MTLE-HS patients (13F, 10 M, see Table 1) who underwent epilepsy surgical treatment (selective amygdalohippocampectomy or anterior temporal lobectomy) at Neurosurgery Department of Hospital Santo António – Centro Hospitalar e Universitário do Porto (HSA – CHUP) has been analysed. The decision for surgery was taken by HSA multidisciplinary epilepsy team incorporating neurologists, neurosurgeons, neuroradiologists, neurophysiologists and neuropsychologists. All patients were resistant to maximal doses of two or more conventional ASDs used during for > 2 years. Pre-surgical assessment was discussed by the team analysing the results of brain MRI, prolonged video-EEG monitoring, ictal and interictal SPECT, neuropsychological assessment and functional brain MRI, in order to precise the epileptogenic zone and to determine the suitability of the patient for surgical intervention. Surgical specimens of the hippocampus and of the anterior temporal lobe were collected. A complete coronal slice of 0.5 cm thick was removed 3 cm posterior to the tip of the temporal pole. Samples were recovered in ice-cold synthetic CSF (10 mM glucose, 124 mM NaCl, 3 mM KCl, 1 mM MgCl$_2$, 1.2 mM NaH$_2$PO$_4$, 26 mM NaHCO$_3$, 2 mM CaCl^2, pH = 7.40) and immediately cryopreserved in liquid nitrogen. The amount of tissue removed did not differ from the strictly necessary for successful surgical practice. All patients gave written informed consent as stated in Declaration of Helsinki. As for the controls the same temporal lobe region from 10 human autopsies (8 M, 2F; 67.0 ± 10.9 years) were analysed. Tissue was collected by a similar procedure from cadavers, with no known previous history of neurological disease, examined at the Forensics Institute of Porto within a short post-mortem delay (of 4 to 7 h). The collection of autopsy brain material was made accordingly to Decree-Law 274/99, of July 22, published *in Diário da República*–1st SERIE A, No. 169, of 22-07-1999, Page 4522, on the regulation on the ethical use of human cadaveric tissue for research. This work was approved by the ethics committee of the participant institutions.

2.2. Immunohistochemistry

Immunohistochemical staining was performed in 2 μm-thick sections with the mouse monoclonal anti-human HLA-DR alpha-chain clone TAL. 1B5 antibody (Dako, Agilent Technologies, Denmark) and the Novolink Polymer Detection kit procedures (Leica, Biosystems, Cambridge, UK). Heat-mediated antigen target retrieval was performed with 10 mM sodium citrate pH 6. Antibody optimum dilution was determined in a tissue positive control to be 1:400. Slides with replacement of the primary antibody with an antibody of the same immunoglobulin isotype were integrated in each experiment as negative labeling controls.