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Brain inflammation in a chronic epilepsy model: Evolving pattern of the translocator protein during epileptogenesis

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ABSTRACT

Aims: A hallmark in the neuropathology of temporal lobe epilepsy is brain inflammation which has been suggested as both a biomarker and a new mechanistic target for treatments. The translocator protein (TSPO), due to its high upregulation under neuroinflammatory conditions and the availability of selective PET tracers, is a candidate target. An important step to exploit this target is a thorough characterisation of the spatiotemporal profile of TSPO during epileptogenesis.

Methods: TSPO expression, microglial activation, astrocyte reactivity and cell loss in several brain regions were evaluated at five time points during epileptogenesis, including the chronic epilepsy phase in the kainic acid-induced *status epilepticus* (KASE) model (n = 52) and control Wistar Han rats (n = 33). Seizure burden was also determined in the chronic phase. Furthermore, ^{18}F-PBR111 PET/MRI scans were acquired longitudinally in an additional four KASE animals.

Results: TSPO expression measured with in vitro and in vivo techniques was significantly increased at each time point and peaked two weeks post-SE in the limbic system. A prominent association between TSPO expression and activated microglia ($p < 0.001$; r = 0.7), as well as cell loss ($p < 0.001$; r = −0.8) could be demonstrated. There was a significant positive correlation between spontaneous seizures and TSPO upregulation in several brain regions with increased TSPO expression.

Conclusions: TSPO expression was dynamically upregulated during epileptogenesis, persisted in the chronic phase and correlated with microglia activation rather than reactive astrocytes. TSPO expression was correlating with spontaneous seizures and its high expression during the latent phase might possibly suggest being an important switching point in disease ontogenesis which could be further investigated by PET imaging.

1. Introduction

Epilepsy is one of the most common chronic neurological disorders with an estimated prevalence of about 65 million people worldwide (Ngugi et al., 2010). It has a devastating impact on the patients' everyday life as it is characterised by spontaneous recurrent seizures (SRS) due to aberrant neuronal excitation. The medicinal therapies available are purely symptomatic, have significant side effects and are ineffective in up to 30% of the patients. Furthermore, the neurobiological processes that result in (acquired) epilepsy remain unclear, which strongly impedes the development of more potent, targeted and efficient treatments. Interestingly, immune-challenging insults like fever, infection

Abbreviations: 3-D, three-dimensional; CA, cornu ammonis; CT, computed tomography; DG, dentate gyrus; DH, dentate hilus; GFAP, glial fibrillary acid protein; KA, kainic acid; KASE, kainic acid-induced *status epilepticus*; MR, magnetic resonance; MRI, magnetic resonance imaging; OD, optical density; PBS, phosphate buffered saline; PET, positron emission tomography; ROI, region of interest; s.c., subcutaneous; SE, *status epilepticus*; SRS, spontaneous recurrent seizures; TLE, temporal lobe epilepsy; TSPO, translocator protein; vEEG, video-electroencephalography; VOI, volume of interest.

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and trauma, are associated with the acute occurrence of seizures and with a higher risk of developing epilepsy later in life (Ravizza et al., 2011; Vezzani et al., 2011). In addition, recent studies have demonstrated that a vast number of different inflammatory mediators are present in both human as well as preclinical resected epileptic brain tissue. Moreover, insults triggering epileptogenesis can cause an immediate upregulation of inflammatory processes in the affected brain areas (Friedman et al., 2009; Dedeurwaerdere et al., 2012b). As a result, it has been demonstrated that brain inflammation can be regarded as both cause and consequence of epileptic seizures. Given that inflammation may be an important factor in the reorganisation of a normal neuronal network into an epileptic one, this offers a new target for the development of therapies and diagnostic tools. Nevertheless, brain inflammation constitutes both beneficial and detrimental aspects. More specifically, innate defensive mechanisms are believed to safeguard the organism from invading pathogens or insults while chronic inflammation is believed to have a negative impact on disease. Due to this complex nature of inflammation it is of vital importance to understand the inflammation context that is created during disease and to characterise the broad spectrum of molecules and processes involved (Dedeurwaerdere et al., 2012a; Amhaoul et al., 2014). These fundamental insights are necessary to completely understand the role of brain inflammation in the pathophysiology of epilepsy, to fully profit from the potential of anti-inflammatory strategies and to explore the possibilities of using inflammation as a potential biomarker (Amhaoul et al., 2014).

A potential candidate biomarker is the translocator protein (TSPO) (Dedeurwaerdere et al., 2012a). This protein, previously known as the peripheral benzodiazepine receptor, prominently features in brain tissue of patients with temporal lobe epilepsy (TLE). TSPO is normally present in low concentrations in glial cells, but upon activation of these cells, it becomes significantly upregulated. Although this indirect relation between brain inflammation and TSPO has been known for several decades, the actual progression of TSPO expression during epileptogenesis has not been fully described. In addition, its role in disease ontogenesis and progression still remains elusive. Therefore, to disentangle the functional implications of TSPO up to the molecular mechanistic level and to elucidate whether TSPO could be a valid biomarker for epilepsy, firstly requires a clear understanding of the spatial expression pattern of TSPO during the different stages of pathology.

Kainic acid-induced *status epilepticus* (KASE) in rats is a well-established model for TLE, which is the most common and refractory form of focal, acquired epilepsy in adults. In this model, epileptogenesis is triggered by the *status epilepticus* (SE). After a latent period, which is not per se a silent period and cannot be exactly outlined in time, SRS occur. With this study, we firstly characterised the spatiotemporal profile of TSPO *post-mortem* in the rat brain of the KASE model using a cross-sectional study design. Secondly, we assessed the relationship of TSPO with other pathologic hallmarks of the KASE model, namely neuronal cell loss and glial activation. Thirdly, the epileptic outcome, namely SRS, was determined during the chronic period as well as its relationship with TSPO expression. Finally, we followed up TSPO expression longitudinally and three-dimensionally (3-D) using in vivo imaging of TSPO with ^{18}F-PBR111 positron emission tomography (PET).

2. Materials and methods

2.1. Animals

Seven week old male Wistar Han rats (Charles River Laboratories, France) were single housed under a 12 h light/dark cycle, in a temperature and humidity controlled environment. Food and water were available ad libitum. Animals were allowed five days of acclimatisation to the animal facilities before the start of the experiments and were treated in accordance with the guidelines approved by the European Ethics Committee (decree 86/609/CEE) and the Animal Welfare Act (7 USC 2131). All animal experiments were approved (ECD 2014-39) by the ethical committee of the University of Antwerp (Belgium).

2.2. Study design

To study TSPO expression, glial activation and cell loss during epileptogenesis and established epilepsy, control and KASE animals were sacrificed at five crucial time points in disease progression: i.e. 2 days (early phase), 1 week (latent phase), 2 weeks (latent phase), 6 weeks (transition phase) and 3 months (chronic phase) post-SE. At the start of the experiments, the number of animals for the first three time points was 4 to 6 for the control and 6 to 9 for the KASE group. A higher number of animals were included in the groups of the 6 week and 3 month time points as seizure burden by means of video-electroencephalography (vEEG) was assessed in these subjects (control $n = 7$–12; KASE $n = 12$–14). To assess whether the implantation of the electrodes itself would cause brain inflammation, a subset of animals (control $n = 3$, KASE $n = 2$) included in the 3 month time point were not implanted. No differences could be demonstrated between animals that underwent surgery and those who did not for all studied histology variables, and thus were included in the analysis. Upon termination of the experiment, brains were dissected for histological investigation.

A descriptive PET investigation was executed to illustrate the results of the cross-sectional study in vivo and to assess the feasibility of measuring TSPO upregulation longitudinally in rats with a non-invasive, previously validated protocol (Dedeurwaerdere et al., 2012a). For this, four KASE animals were repeatedly scanned 2 and 4 weeks post-SE by means of ^{18}F-PBR111 PET. An additional scan 6 weeks post-SE was only available for two out of the four animals. A control animal was scanned 2 weeks post-sham SE. Magnetic resonance imaging (MRI) scans were taken in parallel for co-registration purposes.

2.3. Induction of SE in the KASE model

The animals were repeatedly administered low-dose subcutaneous (s.c.) injections of kainic acid (KA; initial dose 5 mg/kg — subsequent doses 2.5 mg/kg; A.G. Scientific, USA), as this method has proven both its efficacy and low mortality rate, while controls received saline injections (Dedeurwaerdere et al., 2012a). Importantly, this protocol allows for each individual animal to receive a personalised dose of KA, thus rendering the proconvulsant less lethal.

At SE induction, the rats were aged 7.5 weeks weighing 222 ± 1.8 g for the control animals ($n = 33$) and 224 ± 1.7 g for the KASE group ($n = 56$). About 45 min after the initial KA injection (5 mg/kg, s.c.) repetitive injections of 2.5 mg/kg were given every half-hour unless the rats showed non-convulsive epileptic behaviour. In that case, the injections were delayed and stopped when convulsive seizures started. During the entire induction period, the animals were continuously observed and after 4 h of SE, diazepam (4 mg/kg; NV Roche SA, Belgium) was administered intraperitoneally to arrest the seizures. All, but three KASE animals of the 2 day time point, reached SE after receiving an average dose of 13.6 ± 0.91 mg/kg KA. Control animals received a saline injection. To prevent dehydration, Hartmann's solution (10 ml/kg, s.c.; Viaflo Baxter Healthcare, Belgium) was administered at the end of the procedure. Additional care was taken the days following SE by providing the animals with enriched soft food pellets and Hartmann's solution. One animal died during SE, while two others died the first day post-SE (5.4% mortality rate). These animals belonged to the 6 week time point and were excluded from analysis. In addition, the three animals that did not reach SE were also excluded from the study.

2.4. vEEG

2.4.1. EEG-electrode implantation

This procedure was performed in anaesthetised animals (isoflurane: 5% induction, 2.5% maintenance; Forene, Belgium) two weeks before

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