Experimental Neurology 298 (2017) 13–22

Contents lists available at ScienceDirect

Experimental Neurology

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

Research paper

Scavenging reactive oxygen species inhibits status epilepticus-induced neuroinflammation

Pallavi B. McElroy[a], Li-Ping Liang[a], Brian J. Day[a,b], Manisha Patel[a,*]

[a] Department of Pharmaceutical Sciences, University of Colorado Denver, Aurora, CO, United States
[b] Department of Medicine, National Jewish Health, Denver, CO, United States

ARTICLE INFO

Keywords:
Epilepsy
Reactive oxygen species
Neuroinflammation
Antioxidant
Cytokine
Mammalian target of rapamycin
Microglial activation
Pilocarpine

ABSTRACT

Inflammation has been identified as an important mediator of seizures and epileptogenesis. Understanding the mechanisms underlying seizure-induced neuroinflammation could lead to the development of novel therapies for the epilepsies. Reactive oxygen species (ROS) are recognized as mediators of seizure-induced neuronal damage and are known to increase in models of epilepsies. ROS are also known to contribute to inflammation in several disease states. We hypothesized that ROS are key modulators of neuroinflammation i.e. pro-inflammatory cytokine production and microglial activation in acquired epilepsy. The role of ROS in modulating seizure-induced neuroinflammation was investigated in the pilocarpine model of temporal lobe epilepsy (TLE). Pilocarpine-induced status epilepticus (SE) resulted in a time-dependent increase in pro-inflammatory cytokine production in the hippocampus and piriform cortex. Scavenging ROS with a small-molecule catalytic antioxidant decreased SE-induced pro-inflammatory cytokine production and microglial activation, suggesting that ROS contribute to SE-induced neuroinflammation. Scavenging ROS also attenuated phosphorylation of ribosomal protein S6, the downstream target of the mammalian target of rapamycin (mTOR) pathway indicating that this pathway might provide one mechanistic link between SE-induced ROS production and inflammation. Together, these results demonstrate that ROS contribute to SE-induced cytokine production and antioxidant treatment may offer a novel approach to control neuroinflammation in epilepsy.

1. Introduction

Neuroinflammation is a hallmark of several neurological disorders such as epilepsy, Parkinson's disease, Alzheimer's disease and stroke (Amor et al., 2010; Floyd, 1999). The role of inflammatory processes in the etiology of epilepsy has gained considerable attention in the past decade (Aronica and Crino, 2011; Vezzani et al., 2011). Epilepsy is one of the most common neuronal disorders affecting approximately 1% of the population in the United States. Status epilepticus (SE) is a medical emergency arising due to systemic factors or chemical exposures. SE, like traumatic brain injury, hypoxia-ischemia or infection can result in the development of chronic epilepsy. Various biochemical, physiological and structural changes such as neuronal death, axonal sprouting, neurogenesis, and gliosis are initiated following SE leading to network excitability and spontaneous, recurrent seizures by a process known as epileptogenesis (Loscher and Brandt, 2010). Neuroinflammation occurs in human and experimental TLE and is primarily characterized by robust astrogliosis, microglial activation and the production of cytokines and chemokines (Vezzani, 2014; Vezzani et al., 2012). Key evidence supporting the potential role of inflammatory processes in the epilepsies comes from the demonstration that (1) anti-inflammatory therapies have been shown to exert anticonvulsant effects in drug-resistant human epilepsies (Granata et al., 2003; Riikonen, 2003), (2) several mediators have been found in tissue surgically resected from patients with refractory TLE (Baranzini et al., 2002; Hulkkonen et al., 2004), (3) seizure activity per se can cause the activation of the resident immune cells of the brain or microglia, leading to the production of inflammatory cytokines (Riazi et al., 2010) and (4), spontaneous seizures can also perpetuate chronic inflammation (Vezzani et al., 2012). For example, chemically and electrically-induced seizures have shown increased levels of cytokines in the rodent brain (De Simoni et al., 2000; Lehtimaki et al., 2003; Minami et al., 1991). Additional evidence supporting the role of inflammation in epilepsy comes from studies involving transgenic mice overexpressing interleukin-6 (IL-6) or tumor necrosis factor-α (TNF-α), revealing that chronic inflammation can result in several pathological changes including seizures (Akassoglou et al., 1997; Campbell et al., 1993). Therefore, inflammation is not only a consequence of seizure activity but has also been shown to contribute

* Corresponding author at: 12850 East Montview Boulevard, Aurora, CO 80045, United States.
E-mail address: manisha.patel@ucdenver.edu (M. Patel).

http://dx.doi.org/10.1016/j.expneurol.2017.08.009
Received 10 February 2017; Received in revised form 26 July 2017; Accepted 15 August 2017
Available online 16 August 2017

to epileptogenesis. Given that inflammation plays a key role in epilepsy, it is important to understand the detailed mechanisms underlying SE-induced neuroinflammation and identify novel therapeutic avenues for controlling it. One novel mechanism that has recently been linked to inflammation is the production of reactive species and consequent redox alterations.

Pro-inflammatory processes such as cytokine production can be modulated by ROS and redox status (Bulua et al., 2011; Turrens, 2003). ROS have been examined in the etiology of seizures and epileptogenesis (Rowley and Patel, 2013). ROS play a physiological role in cellular processes via redox signaling as well as a deleterious role via oxidation of cellular macromolecules, leading to cellular dysfunction and death (Andersen, 2004). The importance of ROS and redox status in epilepsy comes from several lines of evidence. First, studies have demonstrated that prolonged seizure activity can cause an increase in superoxide ($O_2\cdot^-$) and hydrogen peroxide from both mitochondria and extracellular sources such as NADPH oxidases (Nox2), leading to oxidative damage to cellular macromolecules and ultimately neuronal damage (Liang et al., 2000; Liang and Patel, 2006; Patel et al., 2005; Williams et al., 2015). Secondly, oxidative stress and alterations to the glutathione redox status have been shown to occur throughout the course of epileptogenesis in experimental models of TLE (Rowley et al., 2015; Ryan et al., 2014; Waldbaum et al., 2010). Additionally, pharmacological scavenging of seizure-induced ROS inhibits neuronal death, improves mitochondrial function and cognitive function in chemoconvulsant models of TLE (Pearson et al., 2015; Rowley et al., 2015). Finally, we have recently demonstrated the occurrence of oxidative and nitrative stress in a mouse model of virus-induced TLE by Theiler's murine encephalomyelitis virus (TMEV) infection, where seizures arise because of inflammation (Bhuyan et al., 2015). Therefore, both inflammation and oxidative stress occur in various animal models of epilepsy. However, whether SE-induced ROS and redox alterations contribute to neuroinflammation is unknown. We hypothesized that SE-induced ROS contribute to pro-inflammatory cytokine production. The following observations support the hypothesis. (1) ROS production is critical to the development of several diseases with a major inflammatory component such as chronic obstructive pulmonary disease (COPD), inflammatory bowel disease, acute pancreatitis and hypertension (Escobar et al., 2012; Rahman and Adcock, 2006; Vaziri, 2008; Zhu and Li, 2012). (2) $O_2\cdot^-$ derived from NADPH oxidases as well as the mitochondrial electron transport chain (ETC) is sufficient to increase the production of pro-inflammatory cytokines (Bulua et al., 2011; Turrens, 2003). (3) Changes in the redox potential of tissues have also been demonstrated to modulate the release of pro-inflammatory cytokines (Iyer et al., 2009).

The goals of this study were to determine 1) the time-course of inflammatory cytokine production following pilocarpine-induced SE, 2) if pharmacological removal of ROS mitigates SE-induced cytokine production and microglial activation and 3) which redox-sensitive signaling pathway that is also involved in inflammation, is affected by removal of seizure-induced ROS. SE sufficient to cause epilepsy resulted in a time- and seizure-dependent upregulation of pro-inflammatory cytokines. Catalytic removal of ROS utilizing a small-molecule antioxidant inhibited SE-induced cytokine production, activation of microglial cells and activation of the mammalian target of rapamycin (mTOR) pathway.

2. Methods

2.1. Reagents

All materials were purchased from Sigma or Fisher Scientific unless otherwise mentioned. Manganese (III) meso-tetrakis (di-*N*-ethylimidazole) porphyrin or Mn^{III}TDE-2-ImP^{5+} (known as AEOL 10150 in the literature) was obtained from Aeolus Pharmaceuticals.

2.2. Induction of status epilepticus (SE)

All animal housing and treatments were conducted in compliance with NIH guidelines and following protocols approved by the Institutional Animal Care and Use Committee at the University of Colorado Anschutz Medical Campus. Adult male Sprague Dawley rats (~250–300 g) were purchased from Harlan Laboratories (Indianapolis, Indiana). After a week of acclimation, rats were randomly assigned to saline or pilocarpine group. Pilocarpine rats were treated with methylscopolamine (1 mg/kg) intraperitoneally (i.p.) to mitigate the peripheral cholinergic effects of pilocarpine, 30 min prior to subcutaneous (s.c.) injection with pilocarpine hydrochloride (340 mg/kg) to induce SE. Diazepam (10 mg/kg) was administered i.p., 90 min after pilocarpine to attenuate SE. Age-matched control rats received scopolamine and saline instead of pilocarpine. Rats were sacrificed by CO_2 inhalation followed by immediate decapitation using a guillotine suitable for adult rats at 6 h (h), 24 h, 48 h, 1 week (wk) and 6 wks following pilocarpine treatment and hippocampi and piriform cortices were dissected out for biochemical analyses.

2.3. Monitoring of behavioral seizures

Behavioral seizures were evaluated by direct observation for 6 h after initial injection and scored according to a modified Racine scale (Racine et al., 1972). Briefly, motor seizure severity was classified as follows: Class III animals displayed forelimb clonus with lordotic posture; class IV animals reared with concomitant forelimb clonus; and class V animals, in addition to having a class IV seizure, fell over. Only rats having at least 3 class III convulsive seizures every hour up to 3 h were included in the study. Direct observation confirmed that animals were having very few seizures 48 h post pilocarpine and no convulsive activity 1 wk. post pilocarpine. Rats were also video monitored for 48 h prior to euthanasia in order to confirm epilepsy at the 6 wk. time-point, by assessing behavioral seizures. Only animals with at least two class IV seizures according to the Racine scale were included in the study as responders. To determine the role of SE in neuroinflammation, cytokines were analyzed in the brains of rats which did not undergo SE (termed as non-responders).

2.4. Catalytic antioxidant administration

Pilocarpine treated rats exhibiting SE (according to the above mentioned criteria), were treated with Mn^{III}TDE-2-ImP^{5+} (5 mg/kg, s.c.) also denoted in the literature as AEOL 10150 or saline for controls, starting 60 min after pilocarpine and every 4 h until sacrifice (approximately 6 injections over a 24 h time period). Mn^{III}TDE-2-ImP^{5+} was dissolved in phosphate buffered saline to achieve a final concentration of 5 mg/ml. Animals were divided into 4 groups: 1) Saline-Saline 2) Pilocarpine-saline 3) Pilocarpine-Mn^{III}TDE-2-ImP^{5+} and 4) Saline-Mn^{III}TDE-2-ImP^{5+}. Saline-Mn^{III}TDE-2-ImP^{5+} group had no difference compared to saline-saline animals (data not shown). Animals were sacrificed at 24 and 48 h for biochemistry and pathology.

2.5. Measurement of oxidative stress markers by HPLC

Glutathione (GSH), glutathione disulfide (GSSG), tyrosine (Tyr) and 3-nitrotyrosine (3NT) assays were performed with an ESA (Chelmsford, MA) 5600 CoulArray HPLC equipped with eight electrochemical cells as previously described in the literature (Hensley et al., 1998; Lakritz et al., 1997; Liang et al., 2007). One hippocampus from each rat was sonicated in 0.1 N ice cold perchloric acid (PCA) and centrifuged at 13,000 rpm for 10 min and supernatants collected. The potentials of the electrochemical cells were set at 400/450/500/570/630/690/830/860 mV vs. Pd. Analyte separation was conducted on a TOSOHAAS (Montgomeryville, PA) reverse-phase ODS 80-TM C-18 analytical column (4.6 cm × 250 cm; 5 μm particle size). The mobile phase was