The Roof is Leaking and a Storm is Raging

Repairing the Blood-Brain Barrier in the Fight Against Epilepsy

Gorter, J.A.; Aronica, E.; van Vliet, E.A.

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The Roof is Leaking and a Storm is Raging: Repairing the Blood–Brain Barrier in the Fight Against Epilepsy

J.A. Gorter, PhD¹, E. Aronica, MD, PhD²,³, and E.A. van Vliet, PhD¹,²

¹ Center for Neuroscience, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, the Netherlands
² Department of (Neuro)pathology, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands
³ Stichting Epilepsie Instellingen Nederland (SEIN), the Netherlands
*Correspondence: E.A. van Vliet, University of Amsterdam, Science Park 904, 1098 XH, Amsterdam, the Netherlands; e-mail: e.a.vanvliet@uva.nl

Abstract
A large body of evidence that has accumulated over the past decade strongly supports the role of both blood–brain barrier (BBB) dysfunction and perivascular inflammation in the pathophysiology of epilepsy. Recent preclinical studies indicate that prolonged seizure- or brain injury-induced BBB dysfunction and subsequent perivascular inflammation may play an important role in post-traumatic epileptogenesis. In turn, perivascular inflammation can further sustain BBB dysfunction. In genetic epilepsies, such as tuberous sclerosis complex and other related epileptogenic developmental pathologies, there is an association between the underlying gene mutation, BBB dysfunction, and perivascular inflammation, but evidence for a causal link to epilepsy is lacking. Future neuroimaging studies might shed light on the role of BBB function in different epilepsies and address the potential for disease modification by targeting both the BBB and perivascular inflammation in acquired and genetic epilepsies.

Keywords
epilepsy, blood-brain barrier, perivascular inflammation, extracellular matrix, biomarker, epileptogenesis, brain injury

Introduction
Research into the role of the blood–brain barrier (BBB) in epilepsy started to accelerate during the last 15 to 20 years with renewed interest in studies that showed BBB disruption in epileptogenic brain tissue. At the same time it has become increasingly clear that (neuro)inflammation may play a role in vascular changes (and vice versa) which both are pro-epileptogenic. This has led to the suggestion that repairing the BBB via targeting perivascular inflammation may provide a new option in the fight against epilepsy.

Blood–Brain Barrier Dysfunction in Epilepsy
The BBB is formed by brain capillary endothelial cells that are surrounded by pericytes, astrocytes, and neurons, together referred to as the neurovascular unit (NVU). This NVU is compromised in various neurological disorders, including epilepsy. Pathological changes of the NVU lead to BBB dysfunction which is most obvious in acquired epilepsy in which epilepsy has developed after an initial insult such as (febrile status epilepticus (SE), traumatic brain injury (TBI), stroke, or other brain insults. Studies in SE, seizure, and TBI models have shown that prolonged seizures or brain injury are accompanied by multiple changes of BBB properties. These changes can be disruptive or nondisruptive.
- The disruptive changes are physical changes associated with BBB leakage, which can be detected using a variety of markers (eg, Evans Blue, fluorescein, or horse-radish peroxidase) and occur at the cellular level. They can consist of pericyte and endothelial damage, structural astrocyte changes, destruction of tight junctions, increased vesicular traffic, and breakdown of the glia limitans.
- The nondisruptive changes usually occur at the molecular level. For example, they include release of cytokines/chemokines and/or enzymes by astrocytic endfeet, endothelial cells and pericytes, changes in expression of influx/eflux carriers and changes in expression of cell adhesion molecules. Release of pro-inflammatory cytokines (eg, interleukin-1 beta...
[IL-1β]) and proteases (eg, matrix metalloproteases) by NVU cells and brain infiltration of leukocytes can subsequently lead to disruptive changes, such as destruction of tight junctions and the extracellular matrix (ECM).6,7

**Time Course of BBB Alterations and Inflammation-Related Events Following Brain Insults**

**Evidence From Molecular/Pathological Studies in Brain Tissue**

Several studies in seizure models have helped to provide insight on the sequence of BBB- and inflammation-related events that occur after prolonged seizures (Figure 1). In these studies, BBB leakage was shown using intravenous injection of Evans blue/fluorescein/albumin or by IgG/albumin immunostainings.5 In general, prolonged seizures are associated with excessive glutamate release, causing cellular stress and dysfunction of NVU cells leading to extravasation of serum proteins, activation of inflammatory and cell adhesion molecules and entry of leukocytes into the brain. Using fluorescent angiography it was recently reported that BBB opening could be detected within 10 minutes after focal cortical seizure onset in the rat. This effect could be blocked by D-AP5, an N-methyl-D-aspartate receptor antagonist, indicating glutamate mediated BBB disruption.8 In a slice culture model under low Mg²⁺ conditions, pericytic injury and increased BBB permeability was measured during recurrent seizure activity.9 Using fluorescein-albumin infusion in the in vitro isolated guinea pig brain, it was revealed that BBB disruption occurs within 5 minutes after bicuculline-evoked seizures while increased IL-1β expression was detected within 1 hour in perivascular astrocytes.10 The latter study nicely shows that seizures can rapidly induce BBB disruption and neuroinflammation independent of blood derived proteins or leukocyte infiltration.

In some instances, BBB damage can also be produced by peripheral inflammation,11 leading to SE and later epilepsy in the pilocarpine model12 or leading to a lower seizure threshold in a mouse gut inflammation model13 Considering the numerous functions of gut microbiota that have been reported, including its influence on BBB integrity and function, this may also become an interesting target.14 Extravasation of blood proteins and increased expression of cell adhesion and inflammatory molecules also occur rapidly in temporal lobe epilepsy models that are characterized by the occurrence of spontaneous seizures after a latent period after chemically or electrically induced SE. For example, increased expression of chemokine/cytokine messenger RNA (mRNA) was detected within 30 minutes after soman15 or pilocarpine-induced SE in rats16 and inflammatory mediators were detected
as early as 2 hours after electrically induced SE.\textsuperscript{17} Neuronal translocation of the cellular stress-related danger signal high-mobility group box 1 (HMGB1) could be detected within 90 minutes, while IL-1β mRNA was increased within 1 to 3 hours after febrile SE in rat pups.\textsuperscript{18} All these events take place well before onset of epilepsy in these models. From the studies mentioned above, the picture emerges that the initial seizure triggers BBB disruption and brain entry of blood proteins that initiate pro-epileptogenic alterations, such as gliosis, neuroinflammation, and structural reorganization (Figure 1). This mechanism and pattern of activation is nicely shown in a series of studies using the albumin brain infusion model\textsuperscript{19} where exposure of this serum protein causes activation of transforming growth factor beta (TGF-β) receptors on astrocytes, which leads to numerous molecular changes that contribute to reduced potassium buffering and increased excitability.\textsuperscript{20} At the same time, seizure-induced glutamate release will also activate neuronal and glial receptors to an extent that it can lead to cell injury and rapid release of danger signals (eg, HMGB1 and heat shock proteins) that induce transcription of inflammatory genes and subsequent dysregulation of various homeostatic processes, which may ultimately lead to epileptic seizures.\textsuperscript{21}

After the initial peak of BBB disruption and surge of inflammatory molecules in the involved brain regions during the first week(s) after the initial injury, structural, and functional reorganization occurs during the following weeks (including, among others, neuronal loss, rewiring, gliosis, neuro- and angiogenesis, changes in receptor-transporter- and ion-channel expression),\textsuperscript{21} which ultimately may result in unstable, seizure-prone neuronal networks. (Figure 1). At the same time, neuronal and vascular inflammation persist, although to a much smaller extent than during the latent phase, leading to subtle BBB leakage and contributing to further seizure progression in a subset of animals with recurrent seizures.\textsuperscript{22} Similarly, BBB disruption and perivascular inflammation are also evident shortly after SE and TBI in humans, as well as in patients with chronic intractable epilepsy.\textsuperscript{23-26} It is important to mention however, that in case of TBI a relatively small percentage of people developed epilepsy after injury (<15%), although this percentage was considerably higher (53%) in a military series after penetrating injuries.\textsuperscript{27,28} This indicates that, next to BBB disruption, other (organizational) processes and/or genetic predisposition also play an important role in whether epilepsy will develop or not.

**Link Between Perivascular Inflammation and BBB Dysfunction in Genetic Epilepsies**

There also seems to be a link between perivascular inflammation and the BBB in nonacquired focal epilepsies, although support for this is mainly based on human brain tissue obtained through surgical resection or postmortem from patients with chronic intractable epilepsy.\textsuperscript{24} For example, tuberous sclerosis complex (TSC) represents the prototypic monogenic disorder of mammalian target of rapamycin (mTOR) pathway dysregulation. Strong expression of genes involved in innate and adaptive immune pathways, as well as with ECM organization has been shown to be an overarching feature of the TSC cortical tuber protein coding transcriptome.\textsuperscript{29} There is also evidence of a prenatal activation of key inflammatory pathways in developing TSC brain lesions,\textsuperscript{30} supporting the role of immune-inflammatory responses in the dynamic changes which over time may contribute to the early epileptogenic processes. Moreover, in a mouse model of TSC, over-activation of pro-inflammatory signaling pathways in astrocytes has been observed before epilepsy onset, pointing to the role of mTOR-mediated inflammatory mechanisms in TSC epileptogenesis.\textsuperscript{31} Accordingly, activation of inflammatory processes occurs in a large spectrum of epileptogenic developmental pathologies, such as focal cortical dysplasias and hemimegalencephaly, linked to germline and somatic mutations in mTOR pathway regulatory genes.\textsuperscript{32} Interestingly, the prominent activation of the innate immune response is associated with BBB alterations, including increased permeability (eg, albumin extravasation, and its uptake in astrocytes). Moreover, the mTOR signaling pathway also plays a role in the regulation of vascular function by different mechanisms affecting vascular endothelial cell function.\textsuperscript{33} Thus, mTOR-dependent effects on BBB function may play a role in epileptogenesis in the large spectrum of epileptogenic developmental pathologies associated with a dysregulation of this pathway and the mechanistic links between mTOR pathway regulatory genes and BBB dysfunction, as well as its relationship with perivascular inflammation. Interestingly, treatment with the mTOR inhibitor rapamycin showed reduced BBB leakage during the chronic phase in experimental models of temporal lobe epilepsy.\textsuperscript{34,35} However, whether this effect is a consequence of seizure suppressing properties of the drug or contributes to a real anti-epileptogenic effect still needs to be evaluated in both acquired and genetic models of epilepsy.

**Evidence From Imaging Studies**

During the last decade, preclinical neuroimaging studies have established that BBB disruption and neuroinflammation can be visualized using contrast-enhanced magnetic resonance imaging (CE-MRI) and microglial positron emission tomography imaging.\textsuperscript{5,36} The CE-MRI studies in rodents showed that BBB leakage was prominent in limbic brain regions within the first week after paraoxon-, kainic acid- or pilocarpine-induced SE\textsuperscript{37-40} and still could be detected several weeks after SE in the piriform cortex.\textsuperscript{37,40} The regions where the enhanced signal could be detected corresponded with the regions where albumin extravasation and gliosis had occurred,\textsuperscript{37-40} indicating the close correlation between BBB disruption and neuroinflammation. Also, neuroimaging studies in patients with epilepsy showed BBB disruption and inflammation in epileptogenic brain regions.\textsuperscript{31} Similarly, BBB disruption and inflammation are also evident in animals and patients early after TBI, which is still present during the chronic phase, weeks-months and even years after the initial injury.\textsuperscript{25,26} Therefore, these
neuroimaging methods may provide biomarkers for epilepsy or treatment response in future clinical studies in patients.35,40-45

**Future Drug Therapies: Answers From Preclinical Studies?**

Examples in preclinical studies of treatments that target inflammation and the BBB suggest that therapies aimed at oxidative stress,46 the IL-1β pathway10 the TGF-β pathway,47 or metalloproteases,48 might be quite promising in protecting or repairing the BBB and to prevent epilepsy, at least in animals. Other future options may be combined therapies or the use of microRNA related molecules that have pleiotropic functions in vascular inflammation.49 Thus, potentially there are numerous options available to target and repair the BBB in order to help to prevent posttraumatic epilepsy, varying from targeting the different cellular components of the NVU and specific inflammatory pathways to different regulators of the tight junction complex or ECM; the preferred option will depend on the timing of intervention and the nature and extent of the initial insult or trauma.

**Declaration of Conflicting Interests**

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