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.

DOI
10.1177/1535759719844750

Publication date
2019

Document Version
Final published version

Published in
Epilepsy currents

Current Review
In Basic Science

Epilepsy Currents
2019, Vol. 19(3) 177-181

DOI: 10.1177/1535759719844750
journals.sagepub.com/home/epi
SAGE

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

J.A. Gorter, PhD[1], E. Aronica, MD, PhD[2,3], and E.A. van Vliet, PhD[1,2]

[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 [1] with renewed interest in studies that showed BBB disruption in epileptogenic brain tissue.[2] 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.[3] 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).[4] 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.[1,5] 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 horseradish peroxidase) and occur at the cellular level.[5] They can consist of pericyte and endothelial damage, structural astrocyte changes, destruction of tight junctions, increased vesicular traffic, and breakdown of the glia limitans.[5]
- 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/efflux carriers and changes in expression of cell adhesion molecules.[5] Release of pro-inflammatory cytokines (eg, interleukin-1 beta

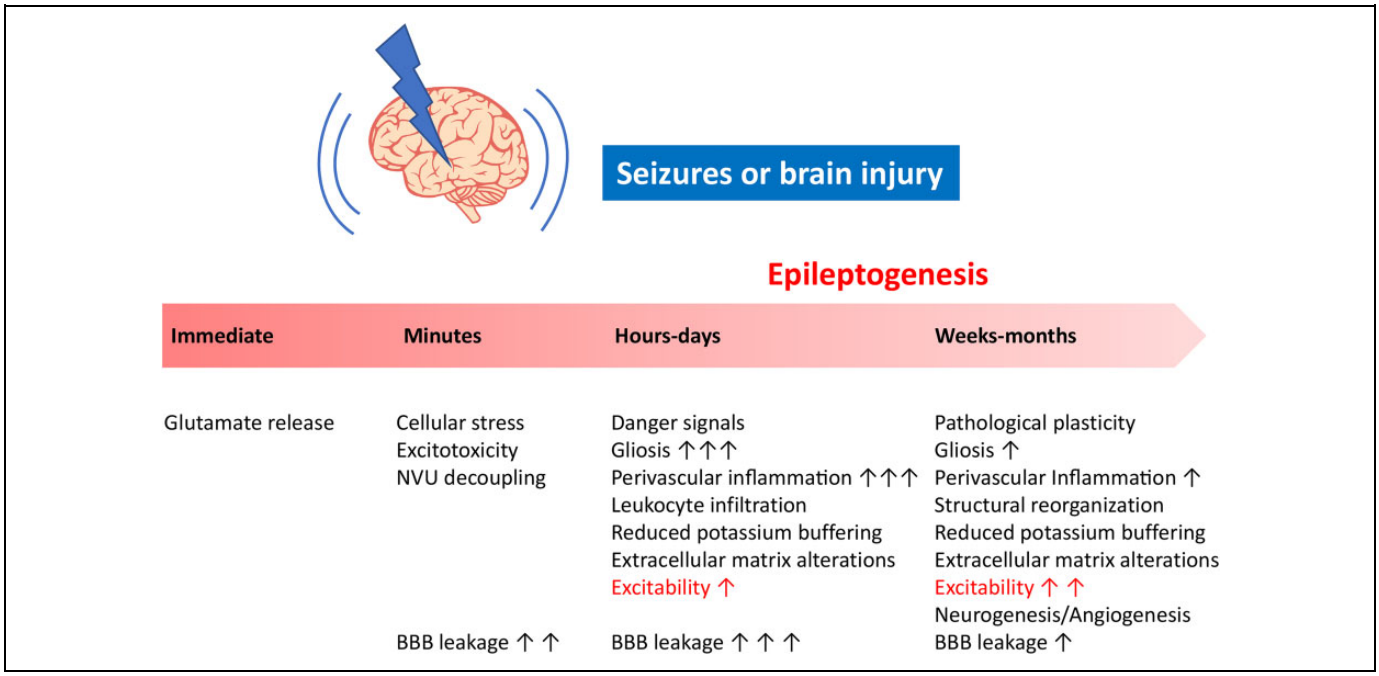

Figure 1. Sequence of events after initial prolonged seizures until the development of epilepsy in animal models. Prolonged seizures are associated with excessive glutamate release that activates NMDA receptors on neuronal/glial cells as well as NVU cells that comprise the BBB. Excessive activation will cause NVU dysfunction leading to rapid BBB leakage (within minutes) and cellular stress which induces the release of danger signals (minutes-hours) that activate toll-like receptors on glial cells resulting in the activation of inflammatory genes and proteases leading to further BBB damage (hours-days). This will ultimately lead to structural and functional reorganization which is accompanied by cell death, aberrant growth, neuro- and angiogenesis, gliosis and inflammation with persistent subtle BBB leakage (weeks-months) which may all contribute to epileptogenesis and seizure progression. BBB, blood–brain barrier; NMDA, N-methyl-D-aspartate; NVU, neurovascular unit.

[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 Mg^{2+} 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 model[12] or leading to a lower seizure threshold in a mouse gut inflammation model[13] 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 soman[15] or pilocarpine-induced SE in rats[16] and inflammatory mediators were detected

as early as 2 hours after electrically induced SE.[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.[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[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.[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.[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),[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.[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.[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.[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.[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.[29] There is also evidence of a prenatal activation of key inflammatory pathways in developing TSC brain lesions,[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.[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.[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.[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.[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.[6,36] 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[37-40] and still could be detected several weeks after SE in the piriform cortex.[37,40] The regions where the enhanced signal could be detected corresponded with the regions where albumin extravasation and gliosis had occurred,[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.[41] 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.[25,26] Therefore, these

neuroimaging methods may provide biomarkers for epilepsy or treatment response in future clinical studies in patients.[25,40-45]

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 pathway[10] 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

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: EA receives grant support from UCB Pharma and served as a consultant for UCB Pharma and Novartis Pharma. We acknowledge funding from the European Union's Seventh Framework Program (FP7/2007-2013) under grant agreement 602102 (EPITARGET; EA, EAvV, JG) and 602391 (EPISTOP; EA), the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement 722053 (EU-GliaPhD; EA) and the Dutch Epilepsy Foundation, project 16-05 (EAvV).

References

1. Gorter JA, van Vliet EA, Aronica E. Status epilepticus, blood-brain barrier disruption, inflammation, and epileptogenesis. *Epilepsy Behav*. 2015;49:13-16.
2. Bauer B, Schlichtiger J, Pekcec A, Hartz AMS. The blood-brain barrier in epilepsy. In: Afawi Z, eds. *Clinical Genetic Aspects of Epilepsy*. London, UK: Intech; 2011;23-54.
3. Vezzani A, French J, Bartfai T, Baram TZ. The role of inflammation in epilepsy. *Nat Rev Neurol*. 2011;7(1):31-40.
4. Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ. Structure and function of the blood-brain barrier. *Neurobiol Dis*. 2010;37(1):13-25.
5. van Vliet EA, Aronica E, Gorter JA. Role of blood-brain barrier in temporal lobe epilepsy and pharmacoresistance. *Neuroscience*. 2014;277:455-473.
6. Fabene PF, Navarro Mora G, Martinello M, et al. A role for leukocyte-endothelial adhesion mechanisms in epilepsy. *Nat Med*. 2008;14(12):1377-1383.
7. Ferrari CC, Depino AM, Prada F, et al. Reversible demyelination, blood-brain barrier breakdown, and pronounced neutrophil recruitment induced by chronic IL-1 expression in the brain. *Am J Pathol*. 2004;165(5):1827-1837.
8. Vazana U, Veksler R, Pell GS, et al. Glutamate-mediated blood-brain barrier opening: implications for neuroprotection and drug delivery. *J Neurosci*. 2016;36(29):7727-7739.
9. Prager O, Kamintsky L, Hasam-Henderson LA, et al. Seizure-induced microvascular injury is associated with impaired neurovascular coupling and blood-brain barrier dysfunction. *Epilepsia*. 2019;60(2):322-336.
10. Librizzi L, Noe F, Vezzani A, de Curtis M, Ravizza T. Seizure-induced brain-borne inflammation sustains seizure recurrence and blood-brain barrier damage. *Ann Neurol*. 2012;72(1):82-90.
11. Varatharaj A, Galea I. The blood-brain barrier in systemic inflammation. *Brain Behav Immun*. 2017;60:1-12.
12. Marchi N, Fan Q, Ghosh C, et al. Antagonism of peripheral inflammation reduces the severity of status epilepticus. *Neurobiol Dis*. 2009;33(2):171-181.
13. Riazi K, Galic MA, Pittman QJ. Contributions of peripheral inflammation to seizure susceptibility: cytokines and brain excitability. *Epilepsy Res*. 2010;89(1):34-42.
14. Logsdon AF, Erickson MA, Rhea EM, Salameh TS, Banks WA. Gut reactions: how the blood-brain barrier connects the microbiome and the brain. *Exp Biol Med (Maywood)*. 2018;243(2):159-165.
15. Svensson I, Waara L, Johansson L, Bucht A, Cassel G. Soman-induced interleukin-1 beta mRNA and protein in rat brain. *Neurotoxicology*. 2001;22(3):355-362.
16. Arisi GM, Foresti ML, Katki K, Shapiro LA. Increased CCL2, CCL3, CCL5, and IL-1beta cytokine concentration in piriform cortex, hippocampus, and neocortex after pilocarpine-induced seizures. *J Neuroinflammation*. 2015;12:129.
17. De Simoni MG, Perego C, Ravizza T, et al. Inflammatory cytokines and related genes are induced in the rat hippocampus by limbic status epilepticus. *Eur J Neurosci*. 2000;12(7):2623-2633.
18. Patterson KP, Brennan GP, Curran M, et al. Rapid, coordinate inflammatory responses after experimental febrile status epilepticus: implications for epileptogenesis. *eNeuro*. 2015;2(5):1-13.
19. Frigerio F, Frasca A, Weissberg I, et al. Long-lasting proictogenic effects induced in vivo by rat brain exposure to serum albumin in the absence of concomitant pathology. *Epilepsia*. 2012;53(11):1887-1897.
20. Heinemann U, Kaufer D, Friedman A. Blood-brain barrier dysfunction, TGFbeta signaling, and astrocyte dysfunction in epilepsy. *Glia*. 2012;60(8):1251-1257.
21. Vezzani A, Fujinami RS, White HS, et al. Infections, inflammation and epilepsy. *Acta Neuropathol*. 2016;131(2):211-234.
22. van Vliet EA, da Costa Araújo S, Redeker S, Aronica E, Gorter JA. Blood-brain barrier leakage may lead to progression of temporal lobe epilepsy. *Brain*. 2007;130(Pt 2):521-534.
23. Broekaart DWM, Anink JJ, Baayen JC, et al. Activation of the innate immune system is evident throughout epileptogenesis and is associated with blood-brain barrier dysfunction and seizure progression. *Epilepsia*. 2018;59(10):1931-1944.

24. van Vliet EA, Aronica E, Gorter JA. Blood-brain barrier dysfunction, seizures and epilepsy. *Semin Cell Dev Biol*. 2015;38:26-34.
25. Dadas A, Janigro D. Breakdown of blood brain barrier as a mechanism of post-traumatic epilepsy. *Neurobiol Dis*. 2019; 123:20-26.
26. Shlosberg D, Benifla M, Kaufer D, Friedman A. Blood-brain barrier breakdown as a therapeutic target in traumatic brain injury. *Nat Rev Neurol*. 2010;6(7):393-403.
27. Annegers JF, Hauser WA, Coan SP, Rocca WA. A population-based study of seizures after traumatic brain injuries. *N Engl J Med*. 1998;338(1):20-24.
28. Ding K, Gupta PK, Diaz-Arrastia R. Epilepsy after traumatic brain injury. In: Laskowitz D, Grant G, eds. *Translational Research in Traumatic Brain Injury*. Boca Raton, FL: CRC Press/Taylor and Francis Group; 2016.
29. Mills JD, Iyer AM, van Scheppingen J, et al. Coding and small non-coding transcriptional landscape of tuberous sclerosis complex cortical tubers: implications for pathophysiology and treatment. *Sci Rep*. 2017;7(1):8089.
30. Prabowo AS, Anink JJ, Lammens M, et al. Fetal brain lesions in tuberous sclerosis complex: TORC1 activation and inflammation. *Brain Pathol*. 2013;23(1):45-59.
31. Zhang B, Zou J, Rensing NR, Yang M, Wong M. Inflammatory mechanisms contribute to the neurological manifestations of tuberous sclerosis complex. *Neurobiol Dis* 2015;80:70-79.
32. Curatolo P, Moavero R, van Scheppingen J, Aronica E. mTOR dysregulation and tuberous sclerosis-related epilepsy. *Expert Rev Neurother*. 2018;18(3):185-201.
33. Galvan V, Hart MJ. Vascular mTOR-dependent mechanisms linking the control of aging to Alzheimer's disease. *Biochim Biophys Acta*. 2016;1862(5):992-1007.
34. van Vliet EA, Forte G, Holtman L, et al. Inhibition of mammalian target of rapamycin reduces epileptogenesis and blood-brain barrier leakage but not microglia activation. *Epilepsia*. 2012;53(7): 1254-1263.
35. van Vliet EA, Otte WM, Wadman WJ, et al. Blood-brain barrier leakage after status epilepticus in rapamycin-treated rats I: magnetic resonance imaging. *Epilepsia*. 2016;57(1):59-69.
36. Scott G, Mahmud M, Owen DR, Johnson MR. Microglial positron emission tomography (PET) imaging in epilepsy: applications, opportunities and pitfalls. *Seizure*. 2017;44:42-47.
37. van Vliet EA, Otte WM, Gorter JA, Dijkhuizen RM, Wadman WJ. Longitudinal assessment of blood–brain barrier leakage during epileptogenesis in rats. A quantitative MRI study. *Neurobiol Dis*. 2014;63:74-84.
38. Breuer H, Meier M, Schneefeld S, et al. Multimodality imaging of blood-brain barrier impairment during epileptogenesis. *J Cereb Blood Flow Metab* 2017;37(6):2049-2061.
39. Bankstahl M, Breuer H, Leiter I, et al. Blood-brain barrier leakage during early epileptogenesis is associated with rapid remodeling of the neurovascular unit. *eNeuro*. 2018;5(3):1-18.
40. Bar-Klein G, Lublinsky S, Kamintsky L, et al. Imaging blood-brain barrier dysfunction as a biomarker for epileptogenesis. *Brain*. 2017;140(6):1692-1705.
41. Veksler R, Shelef I, Friedman A. Blood-brain barrier imaging in human neuropathologies. *Arch Med Res*. 2014;45(8): 646-652.
42. van Vliet EA, Dedeurwaerdere S, Cole AJ, et al. WONOEP appraisal: imaging biomarkers in epilepsy. *Epilepsia*. 2017; 58(3):315-330.
43. Chassidim Y, Vazana U, Prager O, et al. Analyzing the blood-brain barrier: the benefits of medical imaging in research and clinical practice. *Semin Cell Dev Biol*. 2015;38:43-52.
44. Koepp MJ, Arstad E, Bankstahl JP, et al. Neuroinflammation imaging markers for epileptogenesis. *Epilepsia*. 2017;58(suppl 3):11-19.
45. Curran MM, Haddad E, Patterson KP, et al. Epilepsy-predictive magnetic resonance imaging changes following experimental febrile status epilepticus: are they translatable to the clinic? *Epilepsia*. 2018;59(11):2005-2018.
46. Pauletti A, Terrone G, Shekh-Ahmad T, et al. Targeting oxidative stress improves disease outcomes in a rat model of acquired epilepsy. *Brain*. 2017;140(7):1885-1899.
47. Bar-Klein G, Cacheaux LP, Kamintsky L, et al. Losartan prevents acquired epilepsy via TGF-beta signaling suppression. *Ann Neurol*. 2014;75(6):864-875.
48. Rempe RG, Hartz AMS, Soldner ELB, et al. Matrix metalloproteinase-mediated blood-brain barrier dysfunction in epilepsy. *J Neurosci*. 2018;38(18):4301-4315.
49. Iori V, Iyer AM, Ravizza T, et al. Blockade of the IL-1R1/TLR4 pathway mediates disease-modification therapeutic effects in a model of acquired epilepsy. *Neurobiol Dis*. 2017;99:12-23.