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Clinical studies and anti-inflammatory mechanisms of treatments

Jacqueline A. French[*], **Matthias Koepp**[†], **Yvonne Naegelin**[‡], **Federico Vigevano**[§], **Stéphane Auvin**[¶], **Jong M. Rho**[**], **Evan Rosenberg**[*], **Orrin Devinsky**[*], **Peder S. Olofsson**[††], and **Marc A. Dichter**[‡‡]

[*]Comprehensive Epilepsy Center, NYU Langone School of Medicine, New York City, New York, U.S.A [†]Institute of Neurology, University College London, London, United Kingdom [‡]Department of Neurology, University Hospital Basel, Basel, Switzerland [§]Neurology Unit, Department of Neuroscience, Bambino Gesù Children Hospital, Rome, Italy [¶]Pediatric Neurology, Robert Debré University Hospital, Paris, France [**]Alberta Children's Hospital, University of Calgary, Calgary, Alberta, Canada [††]Center for Bioelectronic Medicine, Department of Medicine, Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden [‡‡]Department of Neurology, University of Pennsylvania, Philadelphia, Pennsylvania, U.S.A

Summary

In this exciting era, we are coming closer and closer to bringing an anti-inflammatory therapy to the clinic for the purpose of seizure prevention, modification, and/or suppression. At present, it is unclear what this approach might entail, and what form it will take. Irrespective of the therapy that ultimately reaches the clinic, there will be some commonalities with regard to clinical trials. A number of animal models have now been used to identify inflammation as a major underlying mechanism of both chronic seizures and the epileptogenic process. These models have demonstrated that specific anti-inflammatory treatments can be effective at both suppressing chronic seizures and interfering with the process of epileptogenesis. Some of these have already been evaluated in early phase clinical trials. It can be expected that there will soon be more clinical trials of both "conventional, broad spectrum" anti-inflammatory agents and novel new approaches to utilizing specific anti-inflammatory therapies with drugs or other therapeutic interventions. A summary of some of those approaches appears below, as well as a discussion of the issues facing clinical trials in this new domain.

Keywords

Antiepileptogenesis; Ketogenic diet; Fingolimod; Endocannabinoid system; Vagus nerve stimulation

Address correspondence to Jacqueline A. French, MD, NYU Comprehensive Epilepsy Center, New York, NY 10016, U.S.A. jacqueline.french@nyumc.org and Marc A. Dichter, MD PhD, Department of Neurology, University of Pennsylvania, Philadelphia, PA 19104, U.S.A. dichter@mail.med.upenn.edu.

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Anti-inflammatory treatments may add to the therapeutic armamentarium and help to suppress seizures in epilepsies that are refractory to conventional antiepileptic drugs (AEDs). Although trial methodology for classical AEDs is well established, evaluation of anti-inflammatory treatments for epilepsy has to respect a variety of additional factors, and trial design may become more complex for reasons that will become clear in the sections below. There is expectation and hope that these treatments may even display a true antiepileptogenic effect; a trial to prevent poststroke epilepsy will be discussed in this article. The anti-inflammatory and antiepileptogenic potential of other compounds already in use, like anakinra for rheumatoid arthritis and autoinflammatory diseases and fingolimod for multiple sclerosis, is presented here. Steroids are increasingly in use for the treatment of some epileptic syndromes where an inflammatory cause is suspected. This article presents the rationale behind the administration of these well-known large-spectrum immunosuppressant drugs and weighs their expected benefits against their imminent harms. Cannabinoids modulate the nervous and immune systems by various pathways that will be discussed together with their potential as antiseizure and antiepileptogenic drugs. Nonpharmaceutical therapies for refractory epilepsy such as the ketogenic diet or vagus nerve stimulation also influence the immune system and exert anti-inflammatory effects, which will be addressed in the following chapter.

Clinical Trials of Anti-Inflammatory Agents

(Jacqueline A. French)

Inflammation may be critical to both development and perpetuation of an epileptic focus, and the underlying mechanisms for each may differ.[1] Thus an anti-inflammatory drug may be effective as an antiepileptogenic agent, an anti-ictal agent that suppresses seizures in the chronic state, or both. Moreover, the impact may simply be to suppress seizures, or may be disease-modifying. Clinical trial design will differ substantially depending on the purpose of the trial. Anti-ictal studies are those studies that are performed to determine if the drug can reduce or eliminate ongoing seizures in treatment-resistant patients. These would be similar to studies performed for anti-ictal drugs with other mechanisms, but there may be some special considerations for a drug with anti-inflammatory properties. One issue would be patient selection. To date, preclinical studies have suggested that some degree of inflammation may be present in all patients with chronic epilepsy. However, it is possible that specific etiologies, such as mesial temporal sclerosis (MTS), cortical dysplasia, and tuberous sclerosis have a greater component of inflammation driving ictogenesis.[2] If this is correct, it would be prudent to find patients with these specific etiologies. In addition,, inflammation may be more active at some points in the course of epilepsy than in others. It may not be simple to identify patients who have active inflammation contributing to ictogenesis, and limiting patients to those with specific etiologies may lead to poor recruitment. In future studies, it will be critical to enrich the trial population with responders, to increase effect size. Identification of imaging and/or blood biomarkers will be critical to this effort. Alternative solutions may be “enriched studies” in which patients are initially treated in an open-label fashion, and only responders undergo a randomized clinical trial (RCT), or adaptive trials, in which nonresponders are discontinued early.

Because anti-inflammatory therapies might work in a different way than standard AEDs, the timing of onset and offset of the effect may also be different. Specifically, an anti-inflammatory therapy may take time to exert its effect, and may continue to have an effect after it is withdrawn. An example is VX-765, an anti-inflammatory drug that specifically inhibits interleukin-converting enzyme (ICE), thereby reducing interleukin (IL)-1β biosynthesis and high mobility group box 1 (HMGB1) release, two molecules implicated in seizure mechanisms in experimental models.[1] VX-765 is the only known anti-inflammatory agent that has been used for controlled efficacy trials in chronic focal epilepsy, although these trials were only preliminary.[3] Animal studies showed a delayed start of the antiseizure effect of the anti-inflammatory molecule.[4] This was ignored when designing the first study, which was very short, possibly leading to a failed trial. Only a post hoc analysis showed a probable effect, starting 1–2 weeks after drug initiation. There was also a suggestion of a delayed return to baseline seizure frequency after the drug was withdrawn. Unfortunately, the second study of this drug was terminated after only 10 subjects/arm were enrolled, due to change of priorities on the part of the pharmaceutical company that was developing the molecule. Nonetheless, some very important lessons were learned that can and should be used to inform future trials of anti-inflammatory interventions.

No trials have been done in epilepsy that specifically contain an endpoint to demonstrate disease modification. Disease modification trials have been planned and attempted in other chronic central nervous system (CNS) diseases such as Alzheimer's and Parkinson's disease.[5,6] Trial design options include those that capitalize on delayed start, continued benefit after treatment discontinuation, and active comparison to a non-disease-modifying drug with comparison of slope of improvement over time. These trials may not be easy to perform, and there will need to be some understanding of time to effect and other drug characteristics, to increase the odds of success. Again, biomarkers would be helpful to determine improvement along the way, particularly if there might be substantial delay to effect.

Future directions

Trials to demonstrate antiepileptogenic effects would be the most difficult, and would require careful preclinical studies demonstrating a strong effect of the compound in animal models at relevant doses, duplicated in at least two studies or through a multicenter collaboration. Because most epilepsy risk factors (such as traumatic brain injury and stroke) cause epilepsy in only a subset of affected individuals (typically 10–25%), and epilepsy does not usually develop immediately, trials would need to be long, and would need to recruit a large number of patients. The burden of the therapy should match the potential expected risk reduction, and the trial would have to be planned in such a way that the population is recruitable. Many previous antiepileptogenesis trials have failed due to poor recruitment, or other design flaws.[7] Availability of biomarkers could substantially increase the likelihood of success by either enriching recruitment for patients who will develop epilepsy, or providing an early indicator of benefit.

A Proposal for an Anti-epileptogenesis Trial in Post-stroke Epilepsy

(Matthias Koepp)

Stroke is the third leading cause of death and a major cause of disability in Europe; it affects one in six adults with an estimated 3–6 million stroke cases annually.[8] Individuals who have had a stroke have an increased risk of epilepsy (~10%),[9] with most epilepsy cases occurring within 24 months after stroke. When unprovoked seizures occur, they further impair an already compromised quality of life, worsen the degree of cognitive disability, and are associated with an increased risk of subsequent dementia.[10] There are no treatments to prevent the development of these disabling comorbidities[11] and there are only few effective pharmacologic interventions that facilitate poststroke recovery.[12]

Although progress has been made in understanding the cellular and molecular mechanisms of ischemic tissue damage, neuroprotective and regenerative treatments that could improve outcome in patients recovering from stroke are lacking. Stroke induces a complex cascade of different inflammatory mediators and cytokines, and aging, a risk factor for stroke, further exacerbates neuroinflammatory responses. There is increasing evidence that inflammatory changes in the brain after stroke also promote the development of epilepsy, that is, epileptogenesis, among many other changes through processes triggered by the pro-inflammatory cytokine interleukin (IL)-1β and inhibited by the endogenous IL-1 receptor antagonist (IL-1ra).[1,13,14] Because epileptogenesis is variable and only a minority of patients develop epilepsy following stroke,[15] clinical trials of potentially disease-modifying treatments would benefit greatly from the ability to identify reliably those individuals who are most likely to develop epilepsy after stroke.

Rationale for repurposing an anti-inflammatory compound

Major advances have been made in understanding basic mechanisms of epileptogenesis and epilepsy in a variety of animal models. Among etiologies of epilepsy, which are readily detectable and could permit early interventions, stroke stands out prominently, particularly because patients at-risk and associated epileptogenic mechanisms can be identified within a reasonable time from the insult. One mechanism that has emerged as having primary importance for recovery from stroke and epileptogenesis, is brain inflammation. The available preclinical data support the hypothesis that brain inflammation can play a crucial role not only in promoting epileptogenesis after stroke, but also in sustaining recurrence of seizures once an epileptic condition has become established.[16]

There is evidence indicating that centrally active anti-inflammatory agents can exert neuroprotective effects in animal models of seizure-related cell damage: administration of the human recombinant IL-1ra (anakinra) for 7 days after pilocarpine- or electrically induced status epilepticus (ElectrSE) yielded therapeutic drug levels in brain, and decreased both IL-1β expression in astrocytes and cell loss in rat forebrain.[17] Anakinra given in combination with VX-765, a specific inhibitor of the biosynthetic enzyme of IL-1β, afforded significant neuroprotection in the ElectrSE rat model when given as an intravenous bolus (33 mg/kg, 10 mg/200 μL) followed by sustained subcutaneous infusion with osmotic minipumps (24 mg/day; 80 mg/kg/day) for 1 week starting 3 h after the onset of status

epilepticus. Anakinra treatment was based on previous evidence of neuroprotection in rat and human stroke.[18] Immunohistochemical analysis of brain sections at the end of treatment showed reduction in IL-1β expression in glial cells concomitant with neuroprotection in forebrain areas. However, onset of epilepsy and frequency and duration of seizures assessed 3 months after ElectrSE were not significantly modified. In subsequent studies, however, the combination of anakinra with a cyclooxygenase-2 (COX-2) inhibitor, or with BoxA, a peptide blocking the effects of the inflammatory molecule HMGB1, administered at the time of status epilepticus induction, was able to significantly decrease spontaneous seizures in rats.

Therefore, there is a clear rationale for conducting a proof-of-concept study to test the ability of IL-1 antagonists to inhibit brain inflammation in stroke patients. Demonstration of a central anti-inflammatory action of IL-1 antagonists in the relevant human population is the prerequisite for the design and execution of a randomized controlled trial to determine whether these agents are also effective in achieving inhibition of epileptogenesis, and functional recovery after insult.

Objectives for antiepileptogenesis trial

The development of new, potentially antiepileptogenic therapies has been stagnating, partly due to lack of clinically validated biomarkers to reliably predict who is most likely to develop epilepsy in a short time frame. In fact, currently available tools (e.g., routine magnetic resonance imaging [MRI] and electroencephalography [EEG]), are not accurate predictors for the risk of future seizures, or for the need to start antiepileptic drug treatment after a first unprovoked seizure.

Thus specific objectives for an antiepileptogenesis trial are the following:

1. To evaluate the efficacy of a novel therapeutic strategy in patients following stroke who are at highest risk of developing epilepsy.

   As a first step, it is important to measure the development of a hyperexcitable state following an initial epileptogenic lesion, which underpins the process leading to the occurrence of late unprovoked seizures. Because it is inconceivable to test putative antiepileptogenic drugs over long periods in populations with a very low (<10% over 2 years) risk of a seizure, the challenge is to identify an enriched population of patients with the highest risk of developing epilepsy after stroke. Stroke patients who develop one or more acute symptomatic seizures represent a potentially adequate population for such a trial, as their risk of subsequently developing unprovoked seizures, that is, epilepsy, is about 30% over 2 years.[19]

2. To quantitate the response to anti-inflammatory treatment using imaging, electrophysiologic, and circulating biomarkers.

   The “conditio-sine-qua-non” of an antiepileptogenic treatment targeting poststroke brain inflammation is to demonstrate its capacity to reduce inflammation in the CNS. This may be possible by measuring inflammatory serum biomarkers mirroring brain inflammation, but more specifically by

imaging brain inflammatory changes directly using positron emission tomography (PET) ligands for translocator protein 18 kDa (TSPO), which measure in vivo microglial activation.[20] TSPO is normally only lightly expressed in the brain, but it is drastically upregulated in response to neuroinflammatory stimuli. This upregulation correlates with microglial activation or infiltration of macrophages following stroke.[21]

3. To determine the capacity of biomarkers to predict the development of poststroke epilepsy.

An alternative to evaluating the efficacy of the intervention in preventing epilepsy is to assess the effect of the intervention on appropriate surrogate biomarkers of epileptogenesis with sufficient sensitivity and specificity. Even within the high-risk group, the risk of recurrent seizures will remain below 20% at 1 year. Identification of reliable biomarkers for epileptogenesis would facilitate and shorten targeted trials of novel antiepileptogenic therapies.

Future directions

The quest for appropriate surrogate biomarkers with sufficient sensitivity and specificity is essential for evaluating novel interventions during short-term trials, as well as for identifying populations at higher risk of developing epilepsy than those who can be currently selected. In patients with stroke, the risk of recurrent seizure remains below 10% at 2 years. Thus we propose to focus on an enriched population of patient with stroke and an acute symptomatic seizure, that is, those with the highest risk (~30%) of developing epilepsy.

Fingolimod (FTY720), Is It a Potential Antiepileptic Drug?

(Yvonne Naegelin)

FTY720 has been developed after being isolated from the fungus *Isaria sinclairii.* The synthetic compound fingolimod (Gilenya, 2-amino-2-(2-(4-octylpheyl)ethyl)propane-1,3-diol, $C_{19}H_{33}NO_2$) acts mainly over sequestration of circulating lymphocytes to the lymph nodes without major alterations of their immune functions. It was meant to prevent allograft rejection without inducing a severe immunosuppression but did not show enough immunosuppressive effect in the context of tissue transplantation.[22] It did so, in the context of multiple sclerosis (MS), where it was the first oral drug being approved for relapsing remitting forms, reducing the annual relapse rate by roughly 50%.[23] Acceleration of homing and blocking egress from lymph nodes (over sphingosine 1-phosphate (S1P1) receptor downregulation) is thought to be the main mode of action for its efficacy in MS.[24]

It is well established that inflammation can be a consequence as well as a cause of epilepsy.[25] In contrast to the pathogenesis of MS, where there is a marked activation of the adaptive immune system with infiltrating B and T lymphocytes,[26] brain inflammation in epilepsy is dominated by innate immunity cells including activated microglia, astrocytes, as well as granulocytes and monocytes/macrophages.[27] There seems to be a link between the innate and adaptive immuneresponse,[28,29] possibly explaining why there is some evidence of a scarce presence of adaptive immune cells such as T or B cells in nonautoimmune forms

of epilepsy such as TLE.[27,28,30] In general, the involvement of the adaptive immunity appears to be much more related to the autoimmune forms of epilepsy.[31]

Nevertheless, the two entities might share some common pathways and therefore some treatment approaches.

FTY720 is a potent nonselective agonist at S1P1 and S1P3–5 receptors.[32] FTY720 has a half-life of 6 days and reaches steady-state blood concentrations after 1–2 months of daily dosing. It reduces lymphocytes (homing) within hours after first dose administration, an effect being fully reversible within 6–8 weeks after stopping treatment.[33] Its second most relevant and temporary side effect for clinical applications (negative chronotropy) is caused by targeting S1P1 and S1P3 receptors on atrial myocytes. FTY720 crosses the blood-brain barrier (BBB)[34] to bind S1P receptors located on neural cells in the CNS (S1P1, -3, -5). The S1P2 receptor is located on neural cells as well but not targeted by FTY720. The S1P4 receptor is located only on lymphocytes, but the major effect on lymphocytes is induced by targeting S1P1 receptors on those cells. FTY720 is phosphorylated by sphingosine kinases to FTY720-P.[35] This probably is taking place within the CNS: after oral administration of FTY720, the brain concentrations of FTY720 are 10–27-fold higher than peripheral concentrations.[34] Within the brain, FTY720 and FTY720-P reach nearly the same concentrations.[34]

S1P receptors are also enriched on astrocytes, and these cells are key players in the generation and perpetuation of brain inflammation both in MS and epilepsy as well as in the formation and preservation of the BBB.[36] FTY720-P mediates anti-inflammatory effects on astrocytes as well as extracellular-signal regulated kinase (ERK) phosphorylation by activation of S1P1.[37] The ERK cascade regulates many distinct processes such as proliferation, differentiation, survival, as well as apoptosis of cells. Some data do link ERK activation to epilepsy, but its involvement is still not fully understood. In vitro experiments show a beneficial effect by FTY720-P and FTY720 on endothelial cells, and S1P modulation seems to reduce transmigration of peripheral blood mononuclear cells through the BBB.[38]

In summary, there are various direct effects of FTY720 on the CNS resident cells that go far beyond the homing of peripheral lymphocytes, and most of them are thought to be receptor mediated.[39] These effects may contribute to the therapeutic actions of this drug.

Apart from those receptor-mediated effects, FTY720-P directly binds to histone deacetylase (HDAC), thereby possibly having a direct effect on epigenetic gene regulation.[40] In addition, there seems to be an interaction with other lipids, an inhibition of the cannabinoid receptor CB1, and of phospholipase A2 activity in mast cells.[41]

FTY720 has also been shown to increase brain-derived neurotrophic factor (BDNF) levels and ameliorates symptoms in methyl CpG binding protein 2 (MECP2)-null mice, a model of Rett syndrome.[42] Whether this effect is S1P-mediated is not completely understood. BDNF is upregulated by seizures in animal models, and in brain specimens and blood serum from human pharmacoresistant epilepsy.[43] The current understanding is that BDNF can significantly impact seizures, with either beneficial[44] or detrimental effects being reported.

One recent study has shown both anti-inflammatory and antiepileptogenic effects of FTY720 in a lithium-pilocarpine model of epilepsy in rats.[45]

Because FTY720 ameliorates symptoms of MECP2-null mice,[42] we are performing a clinical study to assess safety and efficacy of oral FTY720 in children with Rett syndrome (FINGORETT). The study (phase I–II) has a focus on safety and was not designed for detecting therapeutic effects on seizures of FTY720 in those children. Nevertheless, information about seizure frequencies before, under, and after treatment are being collected, and EEG studies are recorded on a regular base within the study. The final results will be available in spring of 2017.

Future directions

FTY720 by its multiple modes of action on the immune system, the epigenetic machinery and BDNF signaling may be an interesting candidate as an anti-inflammatory and disease-modifying drug for the treatment of refractory epilepsy. The good penetration across the BBB is another advantage of this drug. Approval and large clinical experience in patients with MS should facilitate and speed up the planning of a clinical trial.

Large Spectrum Anti-inflammatory Treatments: Friend or Foe?

(Federico Vigevano)

The evidence of neuroinflammation and immune mechanism involvement in the genesis of epilepsy is growing,[1,25,46] thus opening the way to immune therapies for treating epilepsy. Immunotherapies are now routinely used in severe epilepsies with potential immune-mediated pathogenesis, such as Rasmussen encephalitis, anti-NMDAR (*N*-methyl-D-aspartate), anti-GAD (glutamic acid decarboxylase) or anti-VGKC (voltage gated potassium channel) complex encephalitis and fever-induced refractory epileptic encephalopathy in school-aged children.[47,48] Beyond these clinical entities, drugs with anti-inflammatory and immunomodulatory actions are considered also in epileptic disorders lacking specific immunologic markers.[49]

Adrenocorticotropic hormone (ACTH) and corticosteroids (prednisone, prednisolone, and hydrocortisone) are widely used in some forms of epilepsies. ACTH and corticosteroids are mostly administered to treat patients with epileptic encephalopathy (EE), a group of severe clinical entities with heterogeneous clinical presentations and variable causes.[50] EE includes prenatal causes like brain malformations, chromosomal or genetic abnormalities and neurocutaneous diseases, perinatal causes such as hypoxic ischemic injuries, and postnatal causes such as vascular or infectious insults.[51] In EE, interictal epileptiform EEG abnormalities play a significant role in generating progressive deterioration in neurologic function.[52] Thus the aim of treatment is not limited to seizure control; more often, the greatest challenge is to improve the child's psychomotor development, a goal that requires suppression or reduction of interictal EEG discharges. With few exceptions, ACTH and corticosteroids are considered first-line agents in all cases of infantile spasms.[53] Therapeutic efficacy has been also reported in other epileptic EE such as Lennox-Gastaut syndrome,

Landau-Kleffner syndrome, continuous spike and wave during sleep (CSWS), and other forms of epilepsy resistant to conventional AEDs.[50]

Little is known on the mechanism of the antiepileptic action of ACTH and corticosteroids, as well as on the pathogenesis of EE. However, various hypotheses have been put forward. At present the most accredited hypothesis is that ACTH and corticosteroids exert their actions through modulation of the hypothalamic-pituitary-adrenal axis. However, the possibility of direct effects on the immune system is also considered. In vitro, ACTH and corticosteroids stimulate the growth of neuroblasts, a property that might be relevant for the treatment of EE occurring in the first year of life. ACTH and corticosteroids may also modulate various neurotransmitter systems and voltage- or receptor-gated ion channels. In particular, ACTH downregulates serotonin 5HT2 receptors in the cerebral cortex, and modulates GABA and dopamine receptors, an effect that, in animal models, is age-dependent.[54]

In the last few years, increasing evidence has accumulated that certain forms of severe epilepsy could have an inflammatory and/or immunologic basis. Hence, the antiseizure effects of ACTH and corticosteroids, as well as immunoglobulins, may depend on their well-known anti-inflammatory and immunosuppressant effects.[55]

ACTH and corticosteroids may cause significant adverse effects. In about two thirds of patients, a transient cerebral atrophy has been described, detectable by brain MRI. This finding may sometimes be a confounding factor in the diagnostic process. Transient dyskinesia with hyperkinetic movements involving face and limbs was observed during treatment with corticosteroids or ACTH. ACTH represents the first-line agent for the treatment of infantile spasms, except for spasms associated with tuberous sclerosis.[56] However, conclusive data supporting the long-term efficacy of ACTH are still lacking in the literature. There are also inadequate data on the optimal dosage and duration of therapy, although short duration and low dose are preferable. The issue of possible efficacy differences between natural and synthetic ACTH is also an object of debate.[57]

Future directions

Immunotherapy trials for the treatment of drug-resistant epilepsy represent a future therapeutic aim. This requires a better definition of how etiopathogenetic immune mechanisms are involved in the mechanisms of epileptogenesis.

The more precise concepts of how inflammation and the immune system may cause epilepsy and/or influence the course of disease will help to refine clinical trials involving the steroids as a class of anti-inflammatory drugs displaying broad effects on the immune system. These trials should also aim at identifying those steroid compounds that may be more effective and have a better adverse event profile.

Anti-Inflammatory Aspects of the Ketogenic Diet and PUFAs

(Stéphane Auvin, Jong M. Rho)

The ketogenic diet (KD) is a high-fat, low-carbohydrate diet characterized by ketonemia, relative hypoglycemia, and high fatty acids levels. The KD is an established treatment for

pharmacoresistant epilepsy, including some inflammation-induced epileptic encephalopathies such as febrile infection-related epilepsy syndrome (FIRES). Although the antiseizure mechanism(s) remain(s) unclear, new potential clinical applications for the KD are rapidly emerging, principally on the basis of its broad neuroprotective actions in various experimental models of neurologic disease, importantly those that significantly involve aberrant inflammatory responses.[58]

Although the KD has been investigated in multiple seizure and epilepsy models, variations on this metabolism-based treatment have also been administered in experimental models of pain, MS, Alzheimer's and Parkinson's diseases, neurotrauma, Autism Spectrum Disorder (ASD), and even malignant brain cancer (Table 1). Furthermore, there is now abundant evidence that the KD possesses anti-inflammatory properties, and specifically, this diet has been shown to decrease proinflammatory cytokine levels after an immune challenge.[59] Although the precise mechanisms underlying such effects in these models remain unclear, there are likely multiple, parallel, and synergistic processes and molecular targets, similar to what has been proposed for epilepsy.[58] For example, polyunsaturated fatty acids (PUFAs), dietary lipids that contain more than one double bond, may play an important mechanistic role in KD action. Systemic PUFA levels rise in response to KD treatment, and have been reported to block epileptiform activity in models in vitro as well as in acutely provoked seizures in rodents. There are two groups of PUFAs: the omega-3 (n-3) and the omega-6 (n-6) PUFAs. This nomenclature refers to the position of the double bond relative to the methyl terminal of the molecule.[60] N-3 PUFAs can decrease the production of inflammatory eicosanoids, cytokines, and reactive oxygen species, and the expression of adhesion molecules. In addition,, n-3 PUFAs act both directly (by replacing arachidonic acid as an eicosanoid substrate and inhibiting arachidonic acid metabolism) and indirectly (by altering the expression of inflammatory genes through effects on transcription factor activation). In addition,, n-3 PUFAs, particularly eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), also exert anti-inflammatory actions, primarily through their hydroxylated metabolites, which include resolvins and docosanoids.[61] Furthermore, PUFAs can bind to and activate peroxisome proliferator-activated receptors (PPARs), including both PPARα and PPARγ. It is notable that synthetic PPARs agonists have been shown to reduce experimentally induced inflammation. This effect is the result of the inhibition of pro-inflammatory pathways involving nuclear factor kappa B (NF-κB), signal transduction and transcription-1, and nuclear factor of activated T cells.[62]

Another KD-related mechanism involved in neuroinflammation is ketone-induced disruption of inflammasome assembly, and ketones such as β-hydroxybutyrate may modulate inflammation through actions on mitochondrial targets.[63] Clearly, with growing evidence that the KD affords anti-inflammatory activity in a variety of animal models and human epileptic conditions (such as FIRES), the concept that inflammation as both a cause of and therapeutic target for epilepsy (and other neurologic conditions) is becoming more valid and worthy of further investigation.[58,64]

Future directions

Preclinical studies should further characterize the mechanisms underlying the anti-inflammatory effects of the KD and its substrates/mediators, not only for epilepsy but also for other neurologic disorders. One intriguing aspect is the potential inflammatory basis of comorbid conditions such as epilepsy and ASD. There is some preliminary clinical[59] and growing experimental evidence[65] that the KD is effective in mitigating core symptoms of ASD, and although the mechanisms underlying such behavioral benefits remain unclear, one intriguing possibility is diet-induced alterations in the gut microbiota, resulting in an anti-microbial-like effect and comparable shifts in specific bacterial abundance seen in humans with autism.[66] Clearly, the KD appears to render beneficial effects in both epilepsy and ASD. Further studies would provide a stronger scientific rationale for much-needed clinical trials for testing unique and complementary treatment approaches for affected patients.

Endocannabinoid System, CB_1R/CB_2Rs, and Inflammation

(Orrin Devinsky)

Δ^9-THC modulates the endocannabinoid system, an endogenous signaling system with hydrophobic ligands *N*-arachidonoyl ethanolamide (anandamide, AEA)[71] and 2-arachidonoyl-glycerol (2-AG).[72] These compounds are produced from postsynaptic membrane phospholipid precursors and released in an activity-dependent, “on-demand” manner. Hydrolysis of endocannabinoids produces arachidonic acid, a precursor for COX-2-mediated production of inflammatory prostaglandins.[73] AEA is degraded by the enzyme fatty acid amide hydrolase (FAAH) into arachidonic acid and ethanolamide,[74,75] and 2-AG is catabolized by monoacylglycerol lipase (MAGL) and α/β-hydrolase domain containing 6 (ABHD6)[76] into arachidonic acid and glycerol.[75–77] Inhibition of the degradative enzymes of AEA and 2-AG shunts lipid mediators away from proinflammatory prostaglandin mediators and toward anti-inflammatory endocannabinoids,[68] thereby reducing neuropathic pain,[78,79] Aβ-mediated inflammation, and oligodendrocyte excitotoxicity.[81] In addition, 2-AG directly inhibits COX-2 function, potentially via nuclear peroxisome proliferator–activated receptor gamma (PPARγ) receptor.[82]

The primary targets for the endocannabinoids are the cannabinoid receptors type-1 (CB_1R) and -2 (CB_2R). CB_1R is the primary CNS receptor, particularly in the hippocampal mossy cell-granule cell synapses, and to a lesser extent, on microglia, astrocytes, and oligodendrocytes.[74] Although implicated primarily in peripheral signaling, CB_2Rs are also expressed in the brain,[83] mediating neuronal excitability[84] and inflammation in microglia.[85] Kainic acid-induced status epilepticus[86] and lipopolysaccharide (LPS)-induced inflammation[87] upregulate CB_1R expression, either as a direct consequence of inflammation-induced sequelae or as a potential compensatory mechanism to limit prolonged hyperexcitability. Supporting a potential anti-inflammatory role of CB_1R/CB2Rs, CB_2R agonism decreases microglial activation, edema, excitotoxicity, oxidative stress, and cell death associated with stroke,[88] germinal matrix hemorrhage,[89] and traumatic brain injury. Furthermore, activation of CB_1Rs[91] and CB_2Rs[92] via synthetic agonists reduced inflammatory nociception in several animal models.

Δ^9-Tetrahydrocannabinol (Δ^9-THC) and inflammation

Both cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (Δ^9-THC) produce net anti-inflammatory effects, although through different target receptor mechanisms (Table 2). Δ^9-THC acts primarily as a partial agonist at CB_1Rs and CB_2Rs on microglia, the primary CNS immune cells. Δ^9-THC reduces LPS-induced inflammation in vitro and in vivo by limiting release of the pro-inflammatory cytokines IL-1β, IL-6, IL-17, tumor necrosis factor α (TNFα), and interferon β (IFNβ), and elevating anti-inflammatory cytokines such as IL-10.[88–94] These effects may be age-specific, however, as treating adolescent mice Δ^9-THC exerts opposite effects when assayed later in life, increasing pro-inflammatory and decreasing anti-inflammatory cytokine release.[95] In other studies, Δ^9-THC limits oxidative stress in LPS-triggered inflammation,[98] reduces nitrite formation following intravitreal NMDA injection,[99] and decreases glutamate neurotoxicity and oxidative stress.[100] Anti-oxidative effects of Δ^9-THC may be independent from, or only partially mediated by, CB_1Rs/CB_2Rs.[100,101] Other proposed anti-inflammatory targets for Δ^9-THC (or related compound Δ^9-tetrahydrocannabivarin, Δ^9-THCV) include transient activation and desensitization of TRP (transient receptor potential) channels TRPA1, TRPV1–4,[102–104] and antagonism of TRPM8,[103] which may regulate postinflammatory Ca^{2+} influx. Similar to endocannabinoids, Δ^9-THC may reduce COX-2 activation via PPARγ,[105] although a Δ^9-THC-mediated, CB_1R-dependent increase in COX-2 and PGE_2[106] may be dose dependent. Collectively, Δ^9-THC primarily exerts anti-inflammatory properties in animal models of inflammation (in vivo) including cerebral artery occlusion–induced ischemia,[107,108] acute palmar inflammation,[109] 5XFAD APP transgenic (Alzheimer's disease model) mice,[106] and the myelin oligodendrocyte glycoprotein (MOG)35-55-induced experimental autoimmune encephalomyelitis model of MS.[101]

Cannabidiol (CBD) and inflammation

CBD has very low affinity at CBRs,[110,111] but acts as an agonist at TRP channels (TRPV1, TRPV2, and TRPA1),[103,104,110,112] 5HT1α receptors,[113] and glycine receptors.[114] CBD is an antagonist at TRPM8 channels,[102] T-type voltage-gated calcium channels,[115] and the G protein-coupled receptor GPR55.[116] CBD may have unique effects on inflammation through dynamic regulation of intracellular calcium stores via multiple, activity-dependent pathways.[117] CBD induces a bidirectional change in intracellular calcium levels that depends on cellular excitability: slightly increasing intracellular [Ca^{2+}] under normal physiologic Ca^{2+} conditions and reducing intracellular [Ca^{2+}] under high-excitability conditions. These changes may be mediated by the mitochondrial Na^+/Ca^{2+} exchanger.[117] CBD also produces biphasic changes in intracellular calcium levels via antagonism of the mitochondrial VDAC1 channel.[118] Furthermore, CBD is an agonist at PPARγ[119] and competitive antagonist at the equilibrative nucleotide transporter (ENT-1), reducing adenosine uptake at baseline[120] and during LPS-induced inflammation in vitro[121] and in vivo.[120]

CBD exerts dynamic changes in intracellular signaling to reduce oxidative stress, as assayed via genome-wide microarray studies. At baseline, CBD treatment in cell cultures targets pathways implicated in oxidative stress and glutathione depletion (e.g., GCN2, PKR, and eIF2a) and nuclear oxidative stress response (e.g. Nrf2).[122,123] Upon LPS stimulation, CBD

triggers Nrf2 activation, inhibits NFκB and AP-1, and activates MAPK, JAK/STAT, and cell cycle regulatory pathways, producing net anti-inflammatory effects and immunosuppression.[94,124] CBD regulates oxidative stress post hypoxic-ischemia[107,113,125] and oxygen-glucose deprivation, in part by reducing LDH efflux, caspase-9 activation, and iNOS expression.[126] Similar to Δ^9-THC, CBD reduces pro-inflammatory cytokines and IFNβ/γ and increases anti-inflammatory cytokines such as IL-4 and IL-10 via a CB1R/CB2R mechanism, potentially through adenosine or PPARγ signaling.[94,101,113,120,122,126,127] This mechanism also limits Th17 differentiation and function, and reduces leukocyte transmigration, partly by downregulating VCAM-1, CCL2, and CCL5, in animal models of MS.[124,128] In addition, CBD increases cell survival and reduces oxidative stress in in vitro and in vivo models of Aβ (1–42)-induced inflammation.[126–129]

Cannabinoids and neuroprotection from seizures

The anti-inflammatory effects of the endocannabinoid system, Δ^9-THC, and CBD may help to explain the neuroprotective effects of cannabinoids in animal models of seizures. In chronic (e.g., pilocarpine-induced) seizure models, synthetic CB_1R agonism[130] or CBD[131,132] administered during the chronic phase reduces neuronal damage and oxidative stress/autophagy. In acute (e.g., kainic acid) preclinical studies, CB_1R activity[133] or elevated endocannabinoid signaling[134,135] provides neuroprotective effects postseizure.

Future directions

CBDs are potentially promising anti-inflammatory drugs. Yet the specific nature, extent, and specificity of their immunomodulatory and immunosuppressive effects remain to be determined. The role of cannabinoids as therapeutic agents against inflammatory and autoimmune disorders, including selected epilepsy syndromes, deserves further study.

Anti-Inflammatory Mechanisms of Vagus Nerve Stimulation

(Peder S. Olofsson)

Accumulating evidence suggests that inflammation and immune system activation may play a role in epileptogenesis and lowering seizure threshold, and that anti-inflammatory treatments improve the disease outcomes.[136–139] Discoveries in bioelectronic medicine, which is the convergence of neuroscience, engineering, computing, and clinical medicine, have revealed that nerve stimulation has the capacity to regulate inflammation, cytokine release, and other immune system functions.[140–143] It is becoming increasingly clear that the immune system no longer can be regarded as fully autonomous, because it is regulated by neural reflex circuits.

The best characterized neural reflex that regulates cytokine release in inflammation is the so called “inflammatory reflex,” in which the vagus nerve plays a key role. Electrical vagus nerve stimulation has been used for treatment of drug-resistant epilepsy since the 1990s[144] and for refractory depression for more than a decade.[145] Vagus nerve stimulators have been implanted in tens of thousands of patients, and few significant adverse effects have been reported.[146–148] The neurophysiologic and molecular mechanisms that underlie the

therapeutic effect of vagus nerve stimulation in epilepsy and depression are, however, not well understood.

Electrical stimulation of the cervical vagus nerve reduces inflammation in a number of experimental inflammatory diseases, ranging from sepsis, over ischemia-reperfusion injury, to experimental arthritis.[141,149] The bridging of neuroscience and immunology has revealed specific molecular targets of neural reflex signals in inflammation.[150] Neurophysiologic and molecular mapping of the efferent arc of the inflammatory reflex has demonstrated that electrical activation of the left cervical vagus nerve triggers signals in the efferent vagus nerve that reach the celiac ganglion where the splenic nerve arises.[151,152] The splenic nerve is required for relaying the signals to the spleen, where choline acetyl-transferase $(ChAT)^+$ T cells, "CD4 T_{ChAT}," which appear in close apposition of splenic nerve endings, release acetylcholine in response to norepinephrine.[153] Acetylcholine subsequently activates α7 nicotinic acetylcholine receptors (α7nAChRs) on innate immune cells, including splenic macrophages, which significantly reduce release of proinflammatory cytokines such as TNF-α in experimental systemic inflammation.[144,154] Vagus nerve stimulation also reduces infiltration of leukocytes in local inflammation,[155] inhibits macrophage activation,[156] and promotes a reparative phenotype of macrophages at sites of injury.[157]

Intriguingly, the discovery that CD4 T_{ChAT} relay neural signals afford a mechanism for providing cholinergic signals to tissues devoid of cholinergic innervation. As an example, most blood vessels are devoid of cholinergic innervation, but vascular endothelial cells express cholinergic receptors. Activation of endothelial cholinergic receptors promotes release of nitric oxide that reduces blood pressure by relaxing vascular smooth muscle cells.[158] CD4 T_{ChAT} are found in murine blood, and mice deficient in CD4 T_{ChAT} show significantly increased blood pressure and their cardiovascular physiology is consistent with an increased systemic vascular resistance.[159] These observations indicate that CD4 T_{ChAT} regulates blood pressure by providing a cholinergic signal to vascular endothelial cells, and it is conceivable that neurotransmitter-releasing immune cells may interact with nerves and organs to regulate physiology in additional ways not yet discovered.

The new insights on reflex control of inflammation and, in particular, on the inflammatory reflex, have spawned clinical trials for treatment of chronic human inflammatory diseases using an implantable vagus nerve stimulator developed originally for treatment of epilepsy.[142] The first reports on its effects in rheumatoid arthritis and inflammatory bowel disease are encouraging, with improved clinical scores and attenuated release of pro-inflammatory cytokines.[160,161] The study population sizes were, however, limited, and results from larger clinical trials that may improve our understanding of the usefulness of vagus nerve stimulation in treatment inflammatory diseases are eagerly awaited.

In light of the accumulating evidence that inflammation plays a role in epileptogenesis and seizure threshold reduction, and the anti-inflammatory effects of vagus nerve stimulation, it is tempting to speculate that one mechanism that underlies the therapeutic effect of vagus nerve stimulation in epilepsy involves attenuation of inflammation. In a recent study, Varvel et al. observed that infiltrating monocytes exacerbate neuronal damage and increase morbidity after status epilepticus.[28] Spleen can deploy a significant portion of the monocytes

recruited to sites of inflammation.[158,162] Vagus nerve stimulation reduces cytokine release from innate immune cells in spleen and may shift macrophage phenotype from pro-inflammatory to reparative.[143] It would be interesting to investigate whether vagus nerve stimulation can reduce epileptogenesis by reducing local neuroinflammation perhaps by local direct effects in the CNS and/or by reducing the pro-inflammatory activity of infiltrating immune cells.

Future directions

Despite the convincing physiologic effects of vagus nerve stimulation in experimental models of inflammatory diseases and the comprehensive understanding of some of the molecular pathways in neural regulation of inflammation, the mechanistic basis for the therapeutic effect of vagus nerve stimulation on epilepsy is currently unclear. Further studies of the neurophysiology and molecular mechanisms that underlie the neural control of immune cell activity both in the periphery and in the CNS are warranted to improve our understanding of the pathogenesis of neuroinflammation and epilepsy.

Conclusions and Future Directions

During the past years, it has been recognized that anti-inflammatory mechanisms play an important role in the antiseizure effect of established therapies for epilepsy, like the KD and vagus nerve stimulation. In addition, a variety of drugs, like CBDs, are under scrutiny for their use as antiseizure drugs, which work through a substantial anti-inflammatory mechanism and which also may have a potential antiepileptogenic effect. When testing these two issues, future trials need to adopt more sophisticated designs with enrollment of “enriched” patients. Treatment of therapy-resistant patients with CBDs or specific anti-inflammatory drugs already approved for other indications, like fingolimod or anakinra, needs to adhere to such a protocol. Prevention of epilepsy after stroke or traumatic brain injury may be another opportune clinical situation for conducting antiepileptogenesis trials. Patterns of adverse events of anti-inflammatory drugs should be closely monitored because they are likely to be different from those observed in classic AEDs. Fostering a network of experimental and clinical research collaborations from academia, industry, and funding institutions is germane for further progress in the translation of preclinical research results into clinical studies for the sake of patients at risk of developing the disease or with difficult-to-treat epilepsy.

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Biography

Dr. Jacqueline French is professor of neurology at NYU Langone Medical Center.

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Key Points

- Specific anti-inflammatory treatments have been effective at suppressing experimental chronic seizures and interfering with epileptogenesis
- Some of these drugs have already been evaluated in early phase clinical trials in pharmacoresistant epilepsies
- Nonpharmaceutical therapies for refractory epilepsy like the ketogenic diet or vagus nerve stimulation influence the immune system
- Novel clinical trial design trials should contain an end-point to demonstrate disease modification

Table 1

Examples of utilization of KD as an anti-inflammatory treatment for various pathologic models

References	Type of diet	Species	Model	Anti-inflammatory effect of KD
[67]	6.6:1 KD 3 weeks before Exp.	Rats	Subcutaneous injection of complete Freund's adjuvant into one hind paw	Decrease both swelling and plasma extravasation
[68]	Made by the lab 2 weeks before Exp.	C57BL/6J Mice	MPTP model	Decrease of activated microglia (Iba 1 staining) Decrease of IL1β, IL-6, TNFα (ELISA of SN)
[69]	6.3:1 KD (Bio-Serv F3666 diet) 7 days before Exp.	C57BL/6 mice	Experimental autoimmune encephalomyelitis S.C. myelin oligodendrocyte glycoprotein (MOG)35–55 peptide + complete Freund's adjuvant (CFA) I.V. 20 ng of pertussis toxin	2-2.5-fold reduction in CNS-derived CD4+ cells and CD11b+ CD45+ cells (macrophage and microglia tendency toward increased CD4+ CD25+ Foxp3+ Treg cells Lymph node & CNS reduction in cytokines (IL-1β, IL-6, TNF-α, IL-12, IL-17) and chemokines (IFN-γ, MCP-1, MIP-1a, MIP-1b)
[70]	6.3:1 KD (Bio-Serv F3666 diet) 4 weeks before Exp.	C57BL/6 mice	Liver and white adipose tissue (WAT)	Liver: Increase of expression of Tnfα, Il-6, Emr1, Cd68, Itgam, Nlrp3 WAT: Decrease of expression of Tnfα, Il-6, Emrl, Cd68, Itgam, Nlrp3
[58]	3:1 KD (Ketocal) 2 weeks before Exp.	Wistar rats	Fever model 50 μg/kg of LPS (*Escherichia coli* 055:B5)	Modulate raise of body temperature Blood: Reduce IL-1β, TNF-α Brain: Reduce IL-1β mRNA

Table 2

Potential anti-inflammatory mechanism of Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD)

	Anti-inflammatory mechanism	References
Δ^9-THC	**(1)** CB_1R/CB_2R partial agonist	137
	(2) ↓pro-inflammatorycytokines (IL-1β, IL-6, IL-17, TNFα, IFNβ) ↑ anti-inflammatorycytokine (IL-10)	93–97
	(3) TRP channels agonist (TRPV1-4, TRPA1), TRPM8 antagonist	102–104
	(4) ↓(or↑) COX-2 activation via PPARγ, dose-dependent	105, 106
CBD	**(1)** Regulation of intracellular Ca^{2+} via mitochondrial Na^+/Ca^{2+} exchanger, or VDAC channel	117, 118
	(2) Inhibition of ENT-1 transporter, ↑ adenosine at A_{2A} receptor	120, 121
	(3) ↓pro-inflammatory cytokines (IL-Iβ, IL-3, IL-6, IL-12, IL-17, TNFα, IFNβ/γ) ↑ anti-inflammatory	94, 101, 113, 120, 121, 125, 127
	cytokines (IL-4, IL-10)	102–104
	(4) TRP channels agonist (TRPV1–4, TRPA1), TRPM8 antagonist	116
	(5) G-protein-coupled-receptor GPR55 antagonist	105
	(6) ↓ COX-2activation via PPARγ	124, 128
	(7) ↓ activation of microglial VCAM-I, CCL2, CCL5 transmigration of leukocytes, (mediated in part by adenosine A_{2A} receptors)	94, 122–124, 126
	(8) ↓ oxidative stress, lipid peroxidation, caspase 3 activation, ROS (iNOS, NO), nuclear stress response (Nrf2→ ↓NFκB, ↑STAT3)	