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Finding a better drug for epilepsy: anti-inflammatory targets

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Abstract

This monograph summarizes one of the sessions of the XI Workshop on Neurobiology of Epilepsy (WONOEP), and provides a critical review of the current state of the field. Speakers and discussants focused on several broad topics: (1) The co-existence of inflammatory processes encompassing several distinct signal-transduction pathways with the epileptogenic process; (2) evidence for the contribution of specific inflammatory molecules and processes to the onset and progression of epilepsy, as well as to epilepsy-related morbidities including depression; (3) the complexity and intricate cross-talk of the pathways involved in inflammation, and the discrete, often opposite roles of a given mediator in neurons vs other cell types. These complexities highlight the challenges confronting the field as it aims to define inflammatory molecules as promising targets for epilepsy prevention and treatment.

Introduction and overview

The Workshop on Neurobiology of Epilepsy (WONOEP), a two-yearly meeting preceding the international epilepsy conferences, has been created by the ILAE Commission on Neurobiology to deal with important areas of basic research in epilepsy. The topic of the 2011 XI workshop was “Finding a better drug for epilepsy”. This critically appraisal is a synthesis of the presentations and questions raised during the discussion of the panel session

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on anti-inflammatory targets. Through this mechanism, this review provides a critical assessment of the field, and the remaining challenges.

The unmet clinical need remains unacceptably high for refractory epilepsy patients. A key goal in the therapy of epilepsy is to improve treatments for controlling spontaneous recurrent seizures in these patients. Another major challenge is **to** develop disease-modifying drugs endowed with anti-epileptogenic properties. Such drugs should prevent the onset of the disease after an epileptogenic injury, or arrest its progression once the disease has already developed. These actions would represent a crucial added value to currently available, mainly symptomatic, therapies. In this context, the putative use of anti-inflammatory drugs in epilepsy has been invoked as a promising therapeutic strategy on the basis of the accumulating experimental and clinical evidence linking brain inflammation to epilepsy in a causal and reciprocal relationship (Fabene, et al. 2010, Vezzani, et al. 2010, Vezzani, et al. 2011b). Indeed, proof-of-concept studies evaluating compounds with clinical potential, which are directed at inflammatory targets are emerging (reviewed in Vezzani et al., 2011b; (Vezzani, et al. 2010).

The presence of various inflammatory mediators in experimental and human epileptic tissue and serum has been well described (Fabene, et al. 2008, Friedman, et al. 2009, Dube, et al. 2010, Vezzani, et al. 2011b). In addition, epileptogenic insults including trauma, ischemia/hypoxia, fever and infection as well as recurrent seizures, have been shown to cause rapid onset inflammatory processes in the brain regions affected by the epileptogenic event. Inflammatory processes are found during the period preceding the onset of frank epilepsy (spontaneous seizures) in experimental models, raising the potential of their role in this pathological process (Dube, et al. 2010, Friedman & Kaufer 2011, Vezzani, et al. 2011b). Several specific inflammatory mediators can contribute to neuronal network hyperexcitability and decrease seizure threshold. Other actions of inflammatory molecules augment cell loss and influence blood-brain barrier (BBB) permeability (Fabene, et al. 2008, Friedman, et al. 2009, Kim, et al. 2009, Friedman 2011, Vezzani, et al. 2011b), thus contributing to seizures and to the epileptogenic process (Marchi, et al. 2007) (Friedman, et al. 2009, Friedman 2011, Pitkanen & Lukasiuk 2011)). The mechanisms of function of inflammatory mediators induced in the brain after an epileptogenic challenge include both activation of post-translational pathways in neurons which affect their excitability threshold, as well as the more widely recognized transcriptional activation of genes in endothelial cells, glia and neurons (Vezzani, et al. 2011b).

An additional aspect of the role of inflammation in epileptogenesis stems from the growing literature on persisting effects of early life inflammation (Koh, et al. 1999, Choi, et al. 2009, Riazi, et al. 2010). These may include reduced seizure threshold, and vulnerability to seizure-induced cell injury, which is accompanied by enhanced brain inflammation. Thus, early-life inflammation might provide the first ‘hit’ in a two-hit hypothesis of epileptogenesis (Koh, et al. 1999).

Genetic tools have also enriched our understanding of inflammation and epilepsy. The use of transgenic mice that over-express or lack cytokines or COX-2 in astrocytes or in neurons, conditionally lack CD11b, thus suppressing microglia activation, or lack specific endothelial cells adhesion molecules, enables dissection of the role of inflammation in the developmental and adult aspects of network excitability. Whereas such tools provide important information about the role of specific mediators on specific cell types, the confounders of potential developmental and compensatory changes intrinsic to these models remain (Vezzani, et al. 2011a).

The evolution of epilepsy itself offers an analogous complexity: we must consider that the beneficial effects obtained by blockade of specific inflammatory processes in the context of established chronic epilepsy may not necessarily influence the dynamic and evolving process of epileptogenesis. Importantly, inflammation is a broad spectrum of molecules and processes and is salubrious or detrimental in specific contexts. These include the extent and duration of injury, the types of tissue, cellular sources and cellular targets of inflammatory mediators, and type of effector molecules released. Within the complexity of this dynamic system, the same molecules playing a detrimental role in chronic spontaneous seizures recurrence may instead have restorative and repair properties in the immediate or proximal phases of epileptogenesis.

As we consider therapeutics, the discovery of biomarkers or surrogate markers is crucial to define populations at risk and to monitor therapeutic efficacy. Imaging techniques, such as Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET), offer promise for monitoring and quantify brain inflammation across pre-clinical and clinical studies. Such sensitive non-invasive and repeatable techniques are instrumental for selecting suitable patients who could best benefit from anti-inflammatory treatments, providing a powerful tool for diagnostic, prognostic and therapeutic purposes (Fabene & Sbarbati 2004, Dedeurwaerdere, et al. 2007, Dube, et al. 2010, Vezzani & Friedman 2011).

In summary, over the past decade, the challenge has been to accumulate supportive information for a potential role of inflammation in epilepsy. This challenge is now behind us. As mentioned in the reviews cited above, there is a plethora of evidence in human tissue and animal models for brain inflammation in epilepsy of various types. The challenge that is facing the field towards “anti-inflammatory” treatment at this point is significantly more daunting: We need to sort out the primary from the secondary phenomena, we need to discern causal contributions to epileptogenesis from ‘innocent bystanders’ and epiphenomena. We need to tease out the complexities of (a) the inflammatory network of molecules and (b) multiple brain cell types and connectivity, and create a precise and useful framework of effectors and their targets. These advancements are required to fully exploit the tremendous potential of anti-inflammatory strategies in epileptogenesis.

Sources and routes for inflammation in epileptogenesis

Inflammation in general is usually induced in response to a noxious stimuli (such as infection or injury), and its purpose is to defend the host against pathogenic threats. In addition, in the western world, misguided immune responses and auto-immune processes are a significant source of inflammation. In the CNS, any tissue injury will induce activation of the innate immune response, which may be followed by activation of adaptive immunity, and can give raise to a gradual and slowly progressing change in the function of the surviving local network. This long-lasting change will be functionally expressed as a reduced threshold of the circuit to generate apparently spontaneous, hypersynchronous, mostly excitatory, neuronal activity (i.e. seizures)(Prince & Tseng 1993). Recent studies indicate that even a mild injury to endothelial cells which will increase vessels permeability within the brain tissue (AKA dysfunction of the blood-brain barrier (BBB)) is sufficient to induce a rapid (within few hours) astroglial response and significant immune response (Friedman, et al. 2009). Indeed, BBB dysfunction is a common finding in many epileptogenic conditions, including *status epilepticus*, traumatic and ischemic brain injuries. An association was reported between vascular alterations and hyperemia in certain brain regions and apoptotic cell death (Fabene, et al. 2003). Furthermore, a positive correlation was described between the extent of BBB opening and the number of spontaneous seizures evoked in rats by electrically-induced *status epilepticus* (van Vliet, et al. 2007). Direct evidence for the involvement of BBB dysfunction in inflammation and epileptogenesis has

been recently established by a series of animal studies in which a long-lasting opening of the BBB in the rat neocortex was shown to result in the delayed appearance of epileptiform activity (for reviews see (Shlosberg, et al. 2010). The mechanisms underlying the gradual development of network hypersynchronicity under the BBB-deprived brain are not entirely clear; however, experimental data support a role for an inflammatory process induced by serum proteins which normally are not found in the adult CNS (e.g. albumin), via transforming growth factor beta (TGFβ) signaling. The rapid activation of astrocytes and microglia following the extravasation of serum proteins into the brain, suggests the BBB as a key regulatory element of the brain innate immune system and the communication between intrinsic brain cells and peripheral immunocompetent cells (Marchi, et al. 2011, Vezzani & Friedman 2011).

Potential molecular targets: cell adhesion molecules

A role for neuronal cell recognition molecules in epileptogenesis, specifically of NCAM, polysialylated NCAM (PAS-NCAM), extra-cellular matrix glycoprotein tenascin-R (TN-R), cadherins and reelin has been postulated. Indeed, in the EL mice, an established model with idiopathic complex partial seizures, which secondarily generalize through the hippocampus (Murashima, et al. 2002), levels of PSA-NCAM, cadherin, TN-R and reelin were significantly increased during early developmental stages (3-7 weeks) and then, decreased at 10 weeks remaining very low thereafter. The downregulation in these proteins was observed before the development of frequent seizures, and contrasted with the unchanged expression of NCAM. The precise ways in which changes in cell recognition molecules in the brain of EL mice interact with inflammation remains obscure (Downer, et al. 2010, Wang & Neumann 2010).

Non-neuronal adhesion molecules, such as ICAM, VCAM, E- and P-selectins have also been demonstrated to play a key role in BBB leakage and in the onset of epileptogenesis induced in mice by pilocarpine (Fabene, et al. 2008). In particular, alpha4 integrin and its ligand VCAM-1 contribute to pathogenic events required for epileptogenesis developing in mice after pilocarpine-induced *status epilepticus* (Fabene, et al. 2008). Furthermore, genetically modified mice deficient in PSGL-1 (Selplg −/−) or in alpha1-3-fucosyltranferases (FucTs) FucT-VII and FucT-IV, which are enzymes required to generate functional selectin-binding carbohydrates, have been reported to be resistant to pilocarpine-induced *status epilepticus*, or to have a reduction in the subsequent number of spontaneous recurrent seizures (Fabene, et al. 2008). It is still not yet resolved if these findings are confined to the pilocarpine model or can be generalized (Zattoni, et al. 2011).

Inflammation in epilepsy co-morbidities

Brain inflammation might also contribute to cognitive defects and depression that commonly are associated with TLE (Mensah, et al. 2006, Dube, et al. 2009, Hecimovic, et al. 2011). Epilepsy-associated depression is severe, and promotes the rate of suicide (Hecimovic, et al. 2011). Therefore specific inflammatory pathways relevant to TLE may also contribute to the evolvement of epilepsy-associated mood and cognitive disorders. Depression-like impairments in the pilocarpine *status epilepticus* model were associated with reduced serotonin output from raphe nucleus and the upregulation of presynaptic serotonin 1-A (5-HT1A) receptors. Selective serotonin reuptake inhibitors (SSRI) fluoxetine exerted no antidepressant effects, whereas treatment with the IL-1 receptor antagonist (IL-1ra) led to the reversal or improvement of performance in tasks considered indicative of depression-like emotions in rodents. Combined administration of fluoxetine and IL-1ra completely abolished all hallmarks of epilepsy-associated depression. SSRI-resistance in epilepsy-associated depression may result from excessive activation of the interleukin-1β (IL-1β)-

mediated signaling; consequently, the use of IL1-β blockers together with SSRI may represent an effective therapeutic approach for SSRI-resistant epilepsy-associated depression (Mazarati, et al. 2010, Pineda, et al. In Press).

High-Mobility Group Box-1 (HMGB1) is released by cell injury or activation and stimulates toll-like receptor 4 (TLR4) and Receptor for Advanced Glycation End Products (RAGE) (Mazarati, et al. 2011, Vezzani, et al. 2011b), which have both been implicated in seizure precipitation and recurrence as well as in memory impairments. Recombinant HMGB1 disrupted object memory encoding in wild type, TLR4 knockout and RAGE knockout animals. Neither TLR4 knockout nor RAGE knockout mice exhibited memory deficits. Blockade of TLR4 in RAGE knockout mice prevented the detrimental effect of HMGB1 on memory. These data suggest that seizure-induced release of HMGB1 may impair non-spatial memory by acting at both TLR4 and RAGE (Mazarati, et al. 2011).

Together these studies support a role for specific proinflammatory pathways in depressive-like behaviors and some cognitive functions.

Biomarkers of inflammation in epilepsy: a role for neuroimaging

Imaging (MRI, PET, ictal SPECT) is widely used for evaluation of patients with epilepsy, including refractory partial epilepsy (Goffin, et al. 2008). Additionally, neuroimaging offers a valuable tool for longitudinal investigations of the development and progression of brain abnormalities accompanying several epilepsy syndromes (Dedeurwaerdere, et al. 2007). Imaging protocols providing biomarkers for brain inflammation will enhance our understanding of the role of inflammation during epileptogenesis. In addition, once anti-inflammatory treatments become available, imaging of brain inflammation would greatly improve evaluation of these novel therapeutic targets in a mechanistic non-invasive way.

Imaging techniques for investigation of the BBB integrity and monocyte infiltration including MRI FLAIR sequence, gadolinium and gadofluorine contrast MRI, and superparamagnetic labeling have been reviewed previously and are not further discussed here (Oude Engberink, et al. 2008, Stoll & Bendszus 2010). So far, the most successful way to visualize microglia activation has been by means of translocator protein (TSPO) specific PET tracers (Winkeler, et al. 2010), which might represent a sensitive imaging approach to monitor brain inflammation in epilepsy. TSPO, also known as peripheral benzodiazepine receptor (PBR) is localized in microglial cells in low amounts under normal healthy conditions. However, when microglia is activated TSPO is expressed highly and hence acts as a biomarker for brain inflammation (Banati 2002). TSPO binding has been detected in surgically resected brain tissue (Johnson, et al. 1992, Kumlien, et al. 1992, Sauvageau, et al. 2002), and *in vivo* by means of PET imaging in TLE patients **(**Hirvonen, et al. 2012**)**. Dedeurwaerdere and colleagues performed a small animal PET study in the kainic acid-induced status epilepticus model using [^{18}F]-PBR111, a radioligand with high specificity for TSPO. They found increased [^{18}F]-PBR111 binding during early epileptogenesis in brain regions involved in seizure generation. These results were validated and confirmed by post-mortem immunohistochemistry of microglia (OX-42) and TSPO autoradiography, confirming the PET imaging of TSPO as a marker of activated microglia. Future efforts should identify novel PET ligands targeting specific pathways in the inflammatory cascade.

Discussion

A. Inflammation: Roles in epileptogenesis and disease progression

As apparent from the introduction, as well as from the presentations and discussion, there is an increasingly clear role for inflammatory molecules in the generation of epilepsy.

Mechanisms exist for initiation and perpetuation of inflammation in the brain, including the local activation of glial cells by the epileptogenic insult (e.g. trauma, fever, infection, etc) or by the recurrence of seizures, and docking and trans-blood vessel invasion of leukocytes into the brain either due to brain parenchymal or to peripheral inflammation, or both, which may contribute to BBB opening (Librizzi, et al. 2007, Fabene, et al. 2008, Marchi, et al. 2011, Vezzani, et al. 2011b, Zattoni, et al. 2011, Librizzi, et al. In Press). Diverse types of inflammatory mediators are synthesized and released in epileptogenic conditions (Gorter, et al. 2006); specific molecules such as IL-1β and TNF-α not only alter neuronal excitability and promote seizures, but appear also to be involved in the plasticity of the network which seems to be essential in the epileptogenic process itself.

There is also an emerging role of inflammatory molecules in disease progression: molecules released from cells injured or activated during an initial insult or during epileptic seizures contribute to inflammatory cascades. An eloquent example is the molecule HMGB-1, released during a variety of cell insults, that activates TLRs. Finally, there is evidence for a contribution of inflammatory processes in the co-morbidities that often accompany epilepsy. A salient one is depression, that arises in close to 50% of individuals with refractory epilepsy (Jackson & Turkington 2005), and comprises specific defects in the function of select neuronal populations. In view of the striking resemblance of cytokine-induced 'sickness behavior' and depression, the discovery of a role for inflammatory cytokines in depression is not surprising.

Thus, in considering the potential utility of anti-inflammatory drugs, four therapeutic goals arise: prevention of epilepsy, management of chronic seizures, management of co-morbidities, and prevention of epilepsy progression.

B. Pathway-specific vs global approaches to inflammation targeting in epilepsy

The presentations and the discussion focused on several distinct molecular and cellular inflammatory pathways. BBB induced activation of TGF-β signaling, TLRs and the agents that activate them, and the downstream consequences, IL-1β–mediatedsignaling, leukocytes and adhesion molecules, and the mTOR-pathway. The latter is discussed in a separate monograph and will not be considered further here.

Evidence in support of contributions of several of these molecular pathways to epilepsy is strong, which raises the possibility that a therapeutic approach might aim to block all inflammation. However, two conceptual problems exist with such an approach. First, the initial trigger or insult likely sets in motion also immune agents and processes that are anti-inflammatory (e.g, IL-ra, IL-10, inhibitors of complement cascade, Insulin Growth Factor, etc). Blocking all of the immune signaling would depress these important 'endogenous anti-inflammatory' agents. In addition, inflammation might contribute to repair and processes that serve to minimize or protect from the major changes in neuronal circuits that promote the emergence of spontaneous seizures. Such a salubrious effect of inflammation is considered to take place in multiple sclerosis, and potentially in other chronic neurodegenerative disorders and spinal cord injury (Schwartz & Shechter 2010). Therefore, a more prudent approach should involve the targeting of a single inflammatory cascade, potentially at several signaling points. An example is blocking IL-1β synthesis via ICE/caspase 1 inhibitors, as well as the interaction of the cytokine with its receptor using IL-1ra, or downstream signaling activation (Vezzani, et al. 2010). The use of such innovative drugs for chronic epilepsy treatment needs to be carefully evaluated regarding side effects and efficient brain delivery as reviewed by (Vezzani, et al. 2010). Whereas the above approach might appear logical, it neglects to consider the strong evidence of cross-talk among distinct inflammatory signaling cascades and pathways. Interference with IL-1β might augment

'compensatory' changes, and potentially worsen the overall outcome. A further confounder, that is only beginning to emerge, is discussed below.

C. Cell-specific consequences of anti-inflammatory approaches

As we consider a potential role for anti-inflammatory strategies in preventing or treating epilepsy, the remarkable complexity of the brain substrate where epilepsy arises must be recognized. Numerous specific cell types, including neurons of various sub-categories and different glial cells express and respond to inflammatory mediators in distinctly different ways. Therefore, a given molecule might be beneficial for a population of cells and be detrimental for another. This was elegantly shown recently for the consequences of deleting neuronal vs astrocytic COX-2, a prostaglandin synthetic enzymes (Serrano, et al. 2011). Similarly, inflammation was recently found to influence neurogenesis differentially in the subventricular zone and subgranular zone of the hippocampus of immature rats (Covey, et al. 2011). Thus, indomethacin, a COX-2 inhibitor, decreased medially situated subventricular zone cells and enhanced proliferation in lateral subventricular zone and hippocampal subgranular zone. The agent also differentially influenced IL-6 expression and microglia migration.

Whereas a role for leukocyte-endothelium interaction in epileptogenesis is suggested, the complete spectrum of the effects of peripheral immune cells is still under active study.

Hence, when addressing means to manipulate selectively distinct inflammatory pathways, these diverse actions should be considered, and the complex total sum of these approaches on epileptogenesis, seizure generation and mechanisms of neuronal repair should guide the therapeutic potential of anti-inflammatory drug candidates.

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