Neuropathology of the Blood-Brain Barrier in Epilepsy: Support to the Transport Hypothesis of Pharmacoresistance

Neuropathology of the Blood-Brain Barrier and Pharmaco-Resistance in Human Epilepsy.

Liu JY, Thom M, Catarino CB, Martinian L, Figarella-Branger D, Bartolomei F, Koepp M, Sisodiya SM. *Brain* 2012;135:3115–3133.

Blood-brain barrier dysfunction is implicated in various neurological conditions. Modulating the blood-brain barrier may have therapeutic value. Progress is hindered by our limited understanding of the pathophysiology of the blood-brain barrier in humans, partly due to restricted availability of human tissue, and because human tissue can only provide limited data about temporal patterns of change. We addressed these important challenges by examining surgically resected brain tissue with various lengths of interval between intracranial depth electrode-related injury and resection, and post-mortem whole brain from patients with drug-sensitive or drug-resistant chronic epilepsy and controls. In this valuable set of resources, we found that: (i) there is a highly localized overexpression of P-glycoprotein in the epileptogenic hippocampus of patients with drug-resistant epilepsy; (ii) this overexpression appears specific to P-glycoprotein and does not affect other transporters; (iii) P-glycoprotein is expressed on the vascular endothelium and end-feet of vascular glia (forming a 'double cuff') in drug-resistant epileptic cases but not in post-mortem controls or surgical epilepsy tissue with electrode-related injuries; (iv) an acute insult from intracranial electrode recording causes localized inflammation, increased blood-brain barrier permeability and structural changes to vasculature detectable for up to at least 330 days and (v) chronic epilepsy is associated with inflammation, enhanced blood-brain barrier permeability and increased P-glycoprotein expression. The occurrence of seizures appears central to P-glycoprotein overexpression. Our findings have potential clinical impact because they directly improve our understanding of blood-brain barrier disruption and transporter expression in humans. In particular, our findings show that the expression of P-glycoprotein in humans is compatible with the inherent assumptions of one current hypothesis of multidrug resistance, and that the specific upregulation of P-glycoprotein expression is likely to be associated with ongoing chronic seizures. There may be a therapeutic window after initial acute injury for the prevention of P-glycoprotein overexpression, and thus this one potential component of drug resistance. Our findings add to the need for careful consideration of the benefit and risks of invasive electroencephalographic recording in surgical evaluation of drug-resistant epilepsy.

Commentary

Approximately one-third of individuals with epilepsy will continue to have seizures despite treatment with antiepileptic drugs (AEDs), and failure of one AED to achieve seizure control often predicts failure of subsequent AED regimens (1). Understanding the mechanisms of drug-resistance in epilepsy is of extreme importance because of potential therapeutic implications. A number of hypotheses exist regarding these mechanisms. The *intrinsic severity hypothesis* argues that since drug resistance is independent of the choice of AED, then it must be related to neurobiological factors that increase disease severity (2). The *target hypothesis* contends that drug-resistance is due to a change in the properties of the AED targets that reduces their sensitivity (3). The *transporter hypothesis* suggests that upregulation of multidrug transporters in the blood-brain barrier limits access of AEDs to their targets in the brain (4). This latter hypothesis is supported by observations that multidrug transporters are overexpressed in capillary endothelial tissue resected from patients with intractable epilepsy (5). Furthermore, modulation of P-glycoprotein was shown to restore sensitivity to the antiepileptic effects of phenobarbital in a rat model of temporal lobe epilepsy (6). Regarding the mechanisms of overexpression of efflux transporters, evidence exists that seizures are associated with P-glycoprotein overexpression—but there is no agreement as to whether AEDs have a direct effect (7). Problems with the existing knowledge about the role of P-glycoprotein in drug-resistance include, among others, lack of appropriate controls in the reports of P-glycoprotein overexpression in tissue resected from patients with epilepsy,

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lack of studies that investigated whole brains for P-glycoprotein expression, and incomplete understanding of the temporal patterns of P-glycoprotein expression in relationship to seizures.

The study by Liu et al. provides strong support for the fundamental assumptions of the transporter hypothesis. The authors studied resected brain tissue from patients with drug-resistant epilepsy and postmortem brain tissue from patients with drug-sensitive and drug-resistant epilepsy. To evaluate the temporal pattern of blood-brain barrier changes associated with injury, the authors also studied resected brain tissue after varying time intervals from depth electrode monitoring. They found localized overexpression of P-glycoprotein but not other multidrug transporters (such as breast cancer resistance protein and multidrug resistance related protein-1), in epileptogenic hippocampi resected from patients with drug-resistant epilepsy. In addition, tissue from patients with drug-resistant epilepsy—but not from those with sustained remission or with depth-electrode-related injury—showed P-glycoprotein overexpression on both the vascular endothelium and vascular glia, forming a "double cuff," a pathologic finding that may be a possible marker of chronic, uncontrolled seizures. Chronic epilepsy was also associated with inflammation and increased permeability of the blood-brain barrier. Interestingly, prior use of phenytoin, carbamazepine, phenobarbital, or valproic acid (considered by the authors to be P-glycoprotein inducers, despite the literature's lack of consensus on this issue) was associated with higher percentage difference of P-glycoprotein immunopositive labeling between the sclerotic hippocampus and control brain regions. Consistent with animal data, higher seizure frequency was also associated with increased immunopositive labeling of hippocampal P-glycoprotein. However, injury related to depth electrode insertion was found to cause localized inflammation and disrupt the integrity of the blood-brain barrier for up to 330 days after the injury—but *without* P-glycoprotein overexpression.

The findings of this study corroborate animal observations that seizures (specifically chronic ongoing seizures) are central to P-glycoprotein overexpression. Confirming these observations in a human study should further encourage attempts to design therapeutic interventions. To illustrate, it is known that excessive glutamate release associated with seizure-related excitatory transmission activates NMDA-receptors, leading to increased intracellular calcium. As a result, the level of phospholipase A2 will increase, mobilizing arachidonic acid from the cell membrane. In the presence of cyclooxygenase-2, arachidonic acid will be converted to prostaglandin E2, which will act on its nuclear receptors, EP1 through EP4, leading to overexpression of P-glycoprotein (7). Targeting this pathway at any level may help modulate P-glycoprotein overexpression. For example, noncompetitive antagonists of the NMDA receptor, such as phencyclidine, may reduce P-glycoprotein expression; unfortunately, this will result in intolerable behavioral adverse events (8). On the other hand, inhibition of cyclooxygenase-2, generally well-tolerated, has been shown in some experiments to reduce P-glycoprotein overexpression and restore the antiepileptic effects of phenobarbital in animals, although other experiments in the kainic acid model suggest that it may *worsen* seizures (7). Prostaglandin E2 receptor antagonists may certainly be a successful therapeutic intervention to prevent or reverse drug resistance. Ongoing research will hopefully shed light on strategies that will have the best balance between efficacy and tolerability.

Animal studies have suggested contradicting evidence regarding which AEDs are transported by P-glycoprotein (7). Liu et al. report increased P-glycoprotein expression in association with prior use of certain AEDs, but their sample size was small and the statistics did not rule out the possible role of confounding variables, such as seizure frequency. Future studies of human blood-brain barrier should explore which AEDs are substrates or inducers of P-glycoprotein and, thus, determine the penetration of what particular AEDs is affected by P-glycoprotein.

This study by Liu et al. also informed ongoing research of in vivo assessment of whole brain P-glycoprotein by neuroimaging. In this regard, animal studies have already established that positron emission tomography (PET) using radiolabeled (R)-[11C] verapamil, a P-glycoprotein substrate, can identify regional changes in P-glycoprotein activity that are induced by seizures (9). Additional studies will deepen our understanding of the temporal pattern of P-glycoprotein expression in relationship to seizures facilitating the emergence of clinically useful interventions. In addition to the fact that modulating P-glycoprotein overexpression in patients presenting with new-onset seizures may lessen chances of drug-resistance, PET imaging of regional P-glycoprotein activity during the surgical evaluation of drug-resistant epilepsy may be of great value in localizing the epileptogenic zone and, thus, improving the surgical outcome.

Finally, since depth electrode insertion was not associated with overexpression of P-glycoprotein, this type of injury did not help characterize the temporal pattern of P-glycoprotein overexpression. That said, the findings did alert clinicians that depth electrode implantation may not be a benign process since it results in chronic inflammatory changes and disruption of the blood-brain barrier. Although experience suggests that these pathologic changes are often clinically inconsequential, more studies should reassess long-term complications of depth electrode monitoring.

by Mohamad Koubeissi, MD

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