

Current Literature

In Clinical Science



P-glycoprotein Expression and Pharmacoresistant Epilepsy: Cause or Consequence?

P-glycoprotein Expression and Function in Patients With Temporal Lobe Epilepsy: A Case-control Study.

Feldmann M, Asselin MC, Liu J, Wang S, McMahon A, Anton-Rodriguez J, Walker M, Symms M, Brown G, Hinz R, Matthews J, Bauer M, Langer O, Thom M, Jones T, Vollmar C, Duncan JS, Sisodiya SM, Koepp MJ. *Lancet Neurol* 2013 Aug;12(8):777-785. doi: 10.1016/S1474-4422(13)70109-1. Epub 2013 Jun 18.

BACKGROUND: Studies in rodent models of epilepsy suggest that multidrug efflux transporters at the blood-brain barrier, such as P-glycoprotein, might contribute to pharmacoresistance by reducing target-site concentrations of antiepileptic drugs. We assessed P-glycoprotein activity in vivo in patients with temporal lobe epilepsy. **METHODS:** We selected 16 patients with pharmacoresistant temporal lobe epilepsy who had seizures despite treatment with at least two antiepileptic drugs, eight patients who had been seizure-free on antiepileptic drugs for at least a year after 3 or more years of active temporal lobe epilepsy, and 17 healthy controls. All participants had a baseline PET scan with the P-glycoprotein substrate (R)-[(11)C]verapamil. Pharmacoresistant patients and healthy controls then received a 30-min infusion of the P-glycoprotein-inhibitor tariquidar followed by another (R)-[(11)C]verapamil PET scan 60 min later. Seizure-free patients had a second scan on the same day, but without tariquidar infusion. Voxel-by-voxel, we calculated the (R)-[(11)C]verapamil plasma-to-brain transport rate constant, K1 (mL/min/cm³). Low baseline K1 and attenuated K1 increases after tariquidar correspond to high P-glycoprotein activity. **FINDINGS:** Between October, 2008, and November, 2011, we completed (R)-[(11)C]verapamil PET studies in 14 pharmacoresistant patients, eight seizure-free patients, and 13 healthy controls. Voxel-based analysis revealed that pharmacoresistant patients had lower baseline K1, corresponding to higher baseline P-glycoprotein activity, than seizure-free patients in ipsilateral amygdala (0.031 vs 0.036 mL/min/cm³; p=0.014), bilateral parahippocampus (0.032 vs 0.037; p<0.0001), fusiform gyrus (0.036 vs 0.041; p<0.0001), inferior temporal gyrus (0.035 vs 0.041; p<0.0001), and middle temporal gyrus (0.038 vs 0.044; p<0.0001). Higher P-glycoprotein activity was associated with higher seizure frequency in whole-brain grey matter (p=0.016) and the hippocampus (p=0.029). In healthy controls, we noted a 56.8% increase of whole-brain K1 after 2 mg/kg tariquidar, and 57.9% for 3 mg/kg; in patients with pharmacoresistant temporal lobe epilepsy, whole-brain K1 increased by only 21.9% for 2 mg/kg and 42.6% after 3 mg/kg. This difference in tariquidar response was most pronounced in the sclerotic hippocampus (mean 24.5% increase in patients vs mean 65% increase in healthy controls, p<0.0001). **INTERPRETATION:** Our results support the hypothesis that there is an association between P-glycoprotein overactivity in some regions of the brain and pharmacoresistance in temporal lobe epilepsy. If this relation is confirmed, and P-glycoprotein can be identified as a contributor to pharmacoresistance, overcoming P-glycoprotein overactivity could be investigated as a potential treatment strategy.

Commentary

Since the 1990s, more than a dozen new medications have been introduced for the treatment of epilepsy. Many of these newer molecules have unique pharmacological mechanisms, and most have significantly improved pharmacokinetic profiles as compared to their predecessors. Despite these advances, however, a frustrating statistic remains: Approximately one-third of patients with epilepsy will ultimately inadequately respond to antiepileptic drug (AED) treatment (1). Pharmacore-

istance is, by international consensus, defined as a failure of two or more AEDs, given in an appropriate manner, to achieve seizure freedom. While the reasons for this drug resistance is no doubt multifactorial, there is a growing body of evidence—derived from both animal models, as well as examinations in brain tissue of patients undergoing surgical resection—that drug resistance might be explained by a failure of AEDs to reach their molecular targets (1).

P-glycoprotein (Pgp) is a member of the ATP-binding cassette proteins. This transmembrane transporter is found in a number of tissues, including endothelial cells of the blood brain barrier (BBB), and functions as an efflux transporter, pumping both xenobiotics and other toxic substrates from the intracellular space back into the capillary lumen. P-glycopro-

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tein (along with other transmembrane efflux pumps) might best be thought of as a defense mechanism, designed to keep potentially harmful molecules from reaching protected areas, such as the brain. Unfortunately, many of our commonly used AEDs are also substrates for this transport protein, at least in some species. Since the mid-1990s, several investigations have suggested Pgp is overexpressed in brain tissue of patients with treatment resistant seizures (2). Early experiments in animal models helped fuel the enthusiasm for the hypothesis that increased expression or upregulation of this (and perhaps other) efflux transporters was responsible for pharmacoresistant epilepsy (3, 4).

Confounding this, however, has been the recognition that marked differences in substrate specificity may exist between both species (i.e., rodent vs human) and tissues (5–8), and the relevance in humans has been questioned (9). Nonetheless, if indeed upregulation of this protein is involved in the development and ultimate expression of pharmacoresistance, how—and perhaps more importantly, why—might this occur in some, but not all, patients? Genetic variability, exposure to certain AEDs, as well as seizures themselves have all been suggested as potential mechanisms.

The gene that encodes for this protein (ABCB1) is highly polymorphic, so it is no surprise that there is great variability in the level of Pgp expression and activity among individuals. Having said that, the available data thus far has failed to show a convincing association between genetic polymorphism of ABCB1 and drug resistant epilepsy (10).

Exposure to certain AEDs themselves, particularly enzyme inducing agents, has been proposed as a potential mechanism for Pgp upregulation. The data supporting this notion is controversial. While some experiments have shown that exposure to certain AEDs increased expression of mRNA or protein content, there are conflicting data regarding increased Pgp functionality in brain endothelial cells (11, 12). Finally, seizure-associated Pgp upregulation has been documented in a number of animal models and likely involves the excitatory transmitter glutamate and NMDA receptors (13). Again, extrapolating experimental data in rodents to the clinic is controversial.

What has been lacking in this story is compelling evidence of the *in vivo* association between Pgp functionality and pharmacoresistance in humans with temporal lobe epilepsy. Recently, Feldmann and colleagues conducted a prospective, case-control study involving patients who were either pharmacoresistant or seizure free for at least 1 year. Control subjects were healthy volunteers. Functionality of Pgp in these groups was assessed using PET and the Pgp substrate (R)-[11C] verapamil along with, in the case of treatment-resistant and healthy subjects, infusion of the Pgp inhibitor tariquidar. Verapamil entry into the brain is limited by Pgp. In this experimental design, lower regional verapamil uptake would suggest increased Pgp activity. Treatment with tariquidar would be expected to inhibit Pgp activity, thus attenuating any observed increase in Pgp activity.

The results of this study are intriguing. At baseline, the patients who had been classified as pharmacoresistant had significantly higher (R)-[11C]verapamil in regions of the hippocampus and temporal lobe as compared to patients deemed seizure free. Moreover, in the pharmacoresistant

patient, (R)-[11C]verapamil correlated inversely with average monthly seizure frequency. No significant differences in (R)-[11C]verapamil were seen between seizure-free patients and healthy controls. While the response was not consistent across all patients, following administration of tariquidar, significant regional differences in increased (R)-[11C]verapamil were seen in pharmacoresistant patients as compared to controls, which the authors suggest is indicative of increased Pgp activity in the ipsilateral hippocampus. Interestingly, the pharmacoresistant patients who showed a lesser increase in (R)-[11C] verapamil uptake as compared to controls following tariquidar, all were taking carbamazepine. None of the other patients were receiving this medication. As previously mentioned, while evidence of carbamazepine induction of Pgp in the brain is lacking, the drug has been shown to increase expression of Pgp in other tissues (14, 15).

Taken together, this novel study would seem to confirm the notion that the efflux transporter Pgp is indeed increased in the drug-resistant patient. The question of causality remains, however. Evidence from Feldman and colleagues could suggest that upregulation of Pgp might be linked to cause, since increases in activity seemed to be localized around the seizure focus. Data from this study also suggested a relationship between seizure frequency and Pgp activity, at least at one point in time. It is tempting, therefore, to conclude that the underlying cause of resistant epilepsy has a simple pharmacokinetic explanation: patients continue to have seizures because the drug could not reach its site of action. The question is, is the simple explanation always the correct answer? It would seem unlikely that these transport proteins evolved at the blood brain barrier for the sole purpose of limiting access to AEDs. Do we find ourselves caught up in our language of “multidrug resistance proteins”? Stated differently, is increased Pgp activity the cause of, or perhaps just a marker for, bad epilepsy? Are patients who became seizure free able to do so because they did not overexpress Pgp at the outset, or did Pgp expression return to normal levels after they became seizure free? This would seem to be a chicken and egg quandary. Which came first? Given that the biological role of Pgp is to remove potentially toxic substances from the intracellular space, one could speculate that upregulation of this transporter is a compensatory mechanism to a compromised blood brain barrier and reflects the overall burden of chronic seizures (12, 16). In any case, the work by Feldmann et al. represents an important step forward in our understanding of the pharmacoresistant patient. Perhaps future *in vivo* investigations will shed light on the relationship between Pgp expression and seizure responsiveness over time.

by Barry E. Gidal, PharmD

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