

## MEANS, MOTIVE, AND OPPORTUNITY: ESTABLISHING CULPABILITY IN PHARMACORESISTANT EPILEPSY

**Inhibition of the Multidrug Transporter P-Glycoprotein Improves Seizure Control in Phenytoin-Treated Chronic Epileptic Rats.** van Vliet EA, van Schaik R, Edelbroek PM, Redeker S, Aronica E, Wadman WJ, Marchi N, Vezzani A, Gorter JA. *Epilepsia* 2006;47: 672–680. **PURPOSE:** Overexpression of multidrug transporters such as P-glycoprotein (P-gp) may play a significant role in pharmacoresistance, by preventing antiepileptic drugs (AEDs) from reaching their targets in the brain. Until now, many studies have described increased P-gp expression in epileptic tissue or have shown that several AEDs act as substrates for P-gp. However, definitive proof showing the functional involvement of P-gp in pharmacoresistance is still lacking. Here we tested whether P-gp contributes to pharmacoresistance to phenytoin (PHT) by using a specific P-gp inhibitor in a model of spontaneous seizures in rats. **METHODS:** The effects of PHT on spontaneous seizure activity were investigated in the electrical post-status epilepticus rat model for temporal lobe epilepsy, before and after administration of tariquidar (TQD), a selective inhibitor of P-gp. **RESULTS:** A 7-day treatment with therapeutic doses of PHT suppressed spontaneous seizure activity in rats, but only partially. However, an almost complete control of seizures by PHT (93%  $\pm$  7%) was obtained in all rats when PHT was coadministered with TQD. This specific P-gp inhibitor was effective in improving the anticonvulsive action of PHT during the first 3–4 days of the treatment. Western blot analysis confirmed P-gp upregulation in epileptic brains (140–200% of control levels), along with approximately 20% reduced PHT brain levels. Inhibition of P-gp by TQD significantly increased PHT brain levels in chronic epileptic rats. **CONCLUSIONS:** These findings show that TQD significantly improves the anticonvulsive action of PHT, thus establishing a proof-of-concept that the administration of AEDs in combination with P-gp inhibitors may be a promising therapeutic strategy in pharmacoresistant patients.

### COMMENTARY

The three pillars of guilt will be instantly recognizable to viewers of television cop shows and courtroom dramas. Means, motive, and opportunity often need to be established to secure a successful prosecution in criminal proceedings. A very similar set of criteria can be considered essential in assessing the validity of biological mechanisms that contribute to disease or shape the individual characteristics of that disease. Just like the detective, it is incumbent on the scientist to establish burden of proof and to provide evidence that is beyond any reasonable doubt.

The issue of pharmacoresistance in epilepsy has received considerable attention in recent years, and the search for mechanisms that might explain why 35% of patients fail to respond to current medications continues apace. A number of plausible hypotheses have been proposed, including inadequate penetration of antiepileptic drugs (AEDs) across the blood–brain barrier; acquired alterations to the structure and/or functionality of ion channels and neurotransmitter receptors that represent the principal targets of AEDs; the narrow pharmacological spectrum, and thereby, inadequacy of current agents; and an inher-

ent resistance, governed by genetic variants of proteins involved in the pharmacokinetics and pharmacodynamics of AED action (1). Of these, the so-called transporter hypothesis, which describes the active extrusion of antiepileptic agents from their intended site of action, is the most extensively researched and documented.

Drug-transporter proteins are expressed throughout the body and control the transfer of endogenous and exogenous molecules across biological membranes. They are predominantly expressed in organs with excretory functions, such as the gastrointestinal tract, liver, and kidney, but also protect sensitive tissues, including the brain, placenta, and testes, from potentially toxic xenobiotics (2). The transporter hypothesis of drug-resistant epilepsy is founded on the premise that overexpression of drug transporting proteins at the blood–brain barrier prevents AEDs from reaching the interstitial space and, thereby, exerting their pharmacodynamic effects. There are, of course, several caveats to this proposition, and these have been elegantly summarized in four specific criteria (3), which represent the “means, motive and opportunity” of the drug-transporter hypothesis.

1. *The mechanism must be detectable in relevant brain tissue:* Initial evidence to support a role for drug-transporter proteins in the causation of refractory epilepsy was

derived from a manuscript by Tishler and colleagues (4) that reported overexpression of the *MDR1* gene, which encodes the drug transporter P-glycoprotein (P-gp), in surgically resected temporal lobe tissue from people with medically intractable epilepsy. Numerous studies have since confirmed this finding and demonstrated an up-regulation of P-gp and related transporters in several other neuropathologies, including focal cortical dysplasia, dysembryoplastic neuroepithelial tumor, and hippocampal sclerosis—all of which are associated with uncontrolled seizures (5). It is interesting to note that the majority of reports suggest that overexpression of transport proteins is confined to the epileptic or lesional area, with relative sparing of adjacent, normal tissue. This observation has important implications for the validity of the hypothesis and would explain why people with non-responsive epilepsy continue to experience CNS-related side effects from their medications.

2. *The mechanism must have appropriate functionality:* If active extrusion by drug-transporter proteins at the blood–brain barrier has functional and clinical relevance to the phenomenon of refractory epilepsy, then the majority of AEDs, if not all of them, must be substrates for one or more transport systems. This fundamental premise has been assessed in a series of experimental investigations employing a variety of techniques and models. Although there remains considerable contention, recent studies in experimental animals appear to suggest that most of the commonly used antiepileptic agents can be transported to some extent by these proteins (6). In the absence of a gold-standard method for the assessment of substrate specificity, there is a common assumption that drug efflux is promiscuous, species-independent, and of sufficient capacity to be nonsaturable at clinically relevant concentrations. On this point, the jury is still out.
3. *The mechanism must be active in drug resistance:* In order to demonstrate the active involvement of transporter proteins in drug responsiveness, it is necessary to establish a link among protein expression, brain drug concentrations, and the efficacy of AEDs. This link has been explored in both experimental seizure models and surgically resected human brain tissue. Up-regulation of P-gp in the rat hippocampus following kainate-induced seizures is associated with a reduction in local phenytoin levels (7), and a direct relationship between the expression of P-gp and concentrations of the primary active metabolite of oxcarbazepine in human brain has been reported (8). These observations are further strengthened by an elegant investigation that demonstrated a correlation between the efficacy of phenobarbital and

levels of P-gp expression in limbic brain regions of animals following status epilepticus induced by electrical stimulation of the basolateral amygdala (9). Not only is the validity of the hypothesis reinforced by these studies, but they also lend support to the notion that seizures can provoke the expression of drug transporters and that this elevated expression, in turn, can restrict brain access of AEDs, completing an apparent vicious cycle.

4. *Overcoming the mechanism counteracts drug resistance:* This final piece of the jigsaw puzzle has been addressed by a handful of experimental and clinical studies (10,11), most notably the recent paper by van Vliet and colleagues, reviewed here. Electrical stimulation of the rat angular bundle was employed to induce an acute status epilepticus and subsequent spontaneous seizures, against which the investigators assessed the efficacy of phenytoin alone and in combination with tariquidar, a novel, selective P-gp inhibitor. When administered alone, phenytoin demonstrated only modest activity, reducing seizure frequency when measured over a 7-day period, without rendering any of the animals seizure-free. In the presence of tariquidar, phenytoin was significantly more effective, with a dramatic reduction in seizure frequency and seizure freedom in five of six animals for up to 4 days. These studies were complemented by Western blot analysis and drug concentration measurements that confirmed elevated expression of P-gp in the ipsilateral hippocampus and entorhinal cortex as well as an increased brain penetration of phenytoin in the presence of tariquidar. When considered together, the multiple findings of this study would appear to offer final and, some might argue, unequivocal confirmation of the multidrug-transporter hypothesis of refractory epilepsy.

The manuscript by van Vliet and colleagues provides support, at least on some level, for all four of the criteria discussed earlier: overexpression of P-gp in a model of epilepsy, evidence to suggest that phenytoin is a substrate for P-gp-mediated extrusion, a demonstration of transporter up-regulation in the causation of drug-resistant seizures, and the reversal of pharmacoresistance by selective inhibition. Of minor concern in this study is the apparently transient effect of P-gp inhibition, the unconvincing efforts to exclude an anticonvulsant effect of tariquidar, the failure to quantify any change in the tolerability profile of phenytoin, and the unusual decision to analyze drug concentrations in the epileptic region alone. Despite these limitations, the experimental evidence offered by van Vliet et al. is relatively compelling. It is perfectly reasonable to conclude that P-gp plays a significant role in mediating resistance to AEDs in animal models of epilepsy and that inhibition of P-gp can

circumvent this mechanism, but whether this phenomenon extends to other drug-transporter proteins or to the clinical arena remains unclear.

Principles of clinical pharmacology dictate that pharmacokinetic barriers to drug action, such as enhanced hepatic metabolism or augmented efflux from the brain, should be surmountable by successive increases in dose. This principle does not apply clinically in the case of intractable epilepsy and, thus far, has not been specifically evaluated in experimental models of refractory seizures. From a clinical standpoint, there is little direct or indirect evidence to support the assertion that AEDs are sufficiently strong substrates for transporter-mediated extrusion from the brain to account for their wholesale lack of efficacy. This premise is supported by data from laboratory studies that suggest compounds, such as phenytoin, are much more effectively transported by rodent P-gp than the human equivalent (12). It is entirely possible that all of this fascinating and at times, convenient evidence applies only to rodents. There is little doubt that the drug-transporter hypothesis of refractory epilepsy has biological plausibility—that is, means, motive, and opportunity have been proven in the laboratory. This assertion is great news for rats with refractory epilepsy but the evidence to suggest that culpability extends to the human species remains largely circumstantial and, as yet, is unlikely to stand up in a court of law.

by Graeme J. Sills, PhD

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