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Editorial

Does P-glycoprotein play a role in pharmacoresistance to antiepileptic drugs?

About one-half of newly diagnosed patients with epilepsy obtain full seizure control with the first antiepileptic drug (AED) tried, and 13% more enter remission with a different drug. The remainder is not likely to obtain satisfactory seizure control with any single drug or drug combination [1]. This vexing problem of pharmacoresistance is familiar to all clinical epileptologists. Many practitioners assume that certain epilepsies, such as catastrophic epilepsies of childhood and some lesional epilepsies including those associated with mesial temporal sclerosis and cortical dysgenesis, are more likely to be refractory to drug treatment, perhaps because the underlying mechanisms of seizure generation in these forms of epilepsy are especially resistant to AEDs. However, in recent years it has become evident that diagnosis is a poor predictor of whether an individual patient will respond to drug treatment [2]. Consequently, other reasons have been sought to explain why a subgroup of patients repeatedly fail one AED after another.

In 1995, Tischler et al. [3] proposed that P-glycoprotein (P-gp), a membrane transporter with a well-recognized role in cancer medicine, could be a cause of medically refractory epilepsy. Since this intriguing proposal, there has been a flurry of research on P-gp, epilepsy and AEDs. How strong is the evidence that P-gp actually is a clinically important cause of AED pharmacoresistance?

Human P-gp, also referred to as MDR1, is a 170-kDa transmembrane glycoprotein that is specifically localized to secretory surfaces of cells in the liver, pancreas, kidney, and intestine where it acts as an energy-dependent pump for many types of lipophilic compounds, including noxious xenobiotics [4]. P-gp transports these substances out of cells into the luminal space so that they can be excreted. In tumor cells P-gp reduces the intracellular concentrations of a wide variety of antineoplastic agents, resulting in “multidrug resistance.”

In addition to its role in the excretion of toxins in peripheral organs, P-gp has been recognized in recent years to be a key component of the blood–brain barrier for lipophilic substances [5]. Hydrophilic (polar) molecules are excluded from entry into the brain parenchyma

by the brain capillary endothelial cell membrane and astrocytic foot processes. In contrast, lipophilic (non-polar) substances cross these membranes easily by passive diffusion. P-gp expressed in the capillary luminal membrane of endothelial cells actively pumps certain of these substances back into blood, thus limiting their brain penetration.

Unlike humans, who have only one drug-transporting P-gp, mice have two, *mdr1a* and *mdr1b*. The substrate specificities of the two isoforms are largely overlapping, although there are preferred drug substrates for each. The two mouse proteins acting together are believed to serve the same function as the single human MDR1 transporter. Mice generated by A.H. Schinkel at The Netherlands Cancer Institute with targeted mutations in the *mdr1* genes have been powerful tools in understanding the role of P-gp in the blood–brain barrier [6]. For example, the opioid loperamide, which is available over the counter for the treatment of diarrhea, has minimal behavioral activity and abuse liability because it does not penetrate the blood–brain barrier. However, in *mdr1a*^{-/-} knockout mice, loperamide exhibits full-blown morphine-like activity [7], demonstrating that P-gp accounts for the drug’s characteristic lack of central nervous system effects at normal therapeutic doses. Experiments in Schinkel’s laboratory indicated that phenytoin, in contrast to loperamide, is a relatively poor substrate for *mdr1a* in vitro and no differences were found between the brain levels achieved in control mice and *mdr1a*^{-/-} mice [7]. More recently, phenytoin brain/plasma concentrations have been shown to be elevated by P-gp inhibitors [8] and in *mdr1alb* double-knockout mice [9], indicating that the drug is transported to some extent. There is also evidence that carbamazepine may be actively transported out of the brain by P-gp [9,10] and it has been suggested that phenobarbital, lamotrigine, and felbamate could also be substrates [5,11].

The discovery that some AEDs may be substrates for P-gp has raised the possibility that P-gp could account for pharmacoresistant epilepsy by nonspecifically limiting brain access to multiple AEDs. In fact, all clinically important AEDs are lipophilic molecules that could

potentially be P-gp substrates. The link between P-gp and pharmacoresistance has been bolstered by studies of brain tissue taken from affected areas in patients with intractable epilepsy. P-gp mRNA levels may be increased by more than 10-fold in brain specimens from such cases [3], and increased expression of P-gp protein has been demonstrated by immunocytochemistry in capillary endothelia and also in astrocytes, which normally do not have detectable levels of P-gp with this technique [12,13]. Moreover, in experimental animals, *mdr1* mRNA and immunoreactivity have been reported to transiently increase after kainate-induced seizures, with a maximal effect at 6 h after kainate treatment [9]. At the time of peak increase, there was a 30% reduction in the phenytoin brain/plasma ratio as determined by microdialysis, indicating that the overexpressed protein is functionally active. In addition, *mdr1* mRNA levels are also persistently elevated in animals rendered chronically epileptic after electrically induced status epilepticus. However, amygdala kindling was not associated with long-lasting increases in P-gp immunoreactivity [14].

Given these tantalizing observations, how confident can we be that P-gp does in fact account for AED pharmacoresistance? For the idea to be fully tenable, many, if not all, commonly used AEDs ought to be P-gp substrates. At present, evidence that a broad range of AEDs are transported by P-gp is lacking. As noted, recent studies support the possibility that phenytoin is a P-gp substrate, albeit a weak one. The conclusions for carbamazepine are more controversial [15]; if P-gp does transport carbamazepine it is very inefficient. Moreover, the article by Sills et al. in a recent issue of *Epilepsy & Behavior* [16] reports that of seven AEDs (phenobarbital, phenytoin, carbamazepine, vigabatrin, lamotrigine, gabapentin, topiramate) examined in *mdr1a*^{-/-} animals and controls, only topiramate behaved like a substrate and a weak one at that. There are several caveats to this study. First, it used a small number of animals and therefore should be considered preliminary. Second, it is conceivable that some or all of the AEDs could be better substrates for *mdr1b*, so that the results might have been different had double-knockout *mdr1alb* mice been used. Third, it is possible that there could be compensatory overexpression of another, unidentified transporter in the knockout animals that takes over the function of the absent *mdr1a* protein. Furthermore, in interpreting this study, one should keep in mind that markedly enhanced P-gp expression in epileptic brain regions could be sufficient to reduce local drug concentrations, even if P-gp does not ordinarily have much influence on overall brain penetration of most of the drugs. In sum, given the current state of knowledge, there is considerable uncertainty as to whether P-gp could be a major cause of nonspecific pharmacoresistance to AEDs.

Nevertheless, as noted by Sills et al., P-gp is not the only efflux pump that may limit drug entry into the central nervous system. For example, within the superfamily of ATP-binding cassette (ABC) transporters, of which P-gp is a member, are seven multidrug resistance-associated proteins (MRPs) that can also account for poor response to cancer chemotherapy [17]. The prototypic MRP, MRP1, is expressed at high levels in the epithelium of the choroid plexus where it is believed to contribute to the blood–cerebrospinal fluid barrier [18]. MRPs may also be present in capillary endothelium and contribute to the blood–brain barrier [19]. Like P-gp, MRPs may be overexpressed in epileptic foci [12,20]. To date, however, there is little direct evidence that MRP1 transports AEDs, although it has been suggested that valproate and possibly phenytoin and carbamazepine could be substrates [12]. In fact, although MRPs can transport some neutral molecules, MRP1 and the other members of the MRP family act mainly to pump organic anion conjugates [17]. There will no doubt be interest in better defining whether the broad substrate range of these transporters encompasses the commonly prescribed AEDs. If these or other drug transport mechanisms can be linked to AED refractoriness, this will open the door to the exciting possibility that inhibitors of these transport systems, or the design of nonsubstrate AEDs, could improve the clinical outcome for patients with pharmacoresistant epilepsy.

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