0026-895X/08/7305-1343–1346$20.00
MOLECULAR PHARMACOLOGY
Vol. 73, No. 5
46680/3335358
*Mol Pharmacol* 73:1343–1346, 2008
*Printed in U.S.A.*


MOL PHARM

*PERSPECTIVE*

# P-Glycoprotein—a Clinical Target in Drug-Refractory Epilepsy?

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Received February 25, 2008; accepted February 26, 2008

## ABSTRACT

ATP-binding cassette transporters such as P-glycoprotein (Pgp), multidrug resistance-associated protein, and breast cancer resistance protein are known to transport a wide range of substrates and are highly expressed in the capillary endothelial cells that form part of the blood-brain barrier. It is noteworthy that P-glycoprotein has been shown to be up-regulated in animal models of refractory epilepsy, and adding a Pgp inhibitor to treatment regimens has been shown to reverse the drug-resistant phenotype. Limited data have suggested a role for Pgp in epilepsy in humans as well. However, few epilepsy drugs have been shown to be transported by Pgp, leading to controversy over whether Pgp actually plays a role in drug-resistant epilepsy. In this issue of *Molecular Pharmacology*, Bauer et al. (p. 1444) demonstrate that glutamate can cause localized up-regulation of Pgp via cyclooxygenase-2 (COX-2) and that this phenomenon can be prevented with COX-2 inhibitors. Localized rather than global up-regulation of Pgp may explain some of the difficulty investigators have had in proving a role for Pgp in epilepsy. The results add new support for future clinical trials targeting Pgp expression in drug-refractory epilepsy.

Overexpression of ATP-binding cassette (ABC) transporters such as P-glycoprotein (ABCB1, Pgp), multidrug-resistance-associated protein (ABCC1, MRP), and breast cancer resistance protein (ABCG2, BCRP) in cancer cells confers a drug-resistant phenotype by transporting a diverse range compounds out of the cell against a concentration gradient (Gottesman et al., 2002). The expression of these transporters in the blood-brain barrier (BBB) and their promiscuous nature has made them attractive targets of study as the potential cause of drug-resistant epilepsy.

In the fields of cancer and epilepsy, we seem to be working on the same puzzle: seeking to establish a case for the role of ABC transporters, such as Pgp, in drug resistance. However, different pieces have fallen into place in each field. In oncology, we know that many of the drugs we use to treat cancer are Pgp substrates, and we can identify overexpression of Pgp in certain cancers; however, we have not been able to convincingly demonstrate significant clinical benefit when we combine Pgp substrate drugs with an inhibitor. In neurology, it seems established that Pgp is up-regulated in refractory epilepsy in animals and humans, and although adding a Pgp inhibitor reverses drug-resistant epilepsy in animal models and (anecdotally) in patients, few epilepsy drugs have been shown to be transported by Pgp.

Fueling the argument that ABC transporters play a role in drug-resistant epilepsy is the nature of the BBB itself. The brain endothelial cells that form the BBB are characterized by expression of several ABC transporters, such as Pgp and ABCG2, and multidrug resistance-associated proteins (MRPs), such as MRP1, -2, -4, and -5 (Deeken and Loscher, 2007). Pgp and ABCG2 have been found to be highly expressed and colocalized in brain microvasculature (Sisodiya et al., 2006). Mice that are deficient in Abcb1a or Abcg2 have increased brain penetration of substrate compounds such as vinblastine (Cisternino et al., 2001) or imatinib (Breedveld et al., 2005), respectively. Thus, the ability of ABC transporters to limit brain penetration of cancer drugs may extend to antiepileptic drugs (AEDs). Such a possibility has led to several studies examining expression of Pgp in animal models of sensitive and resistant epilepsy as well as in patients.

Article, publication date, and citation information can be found at http://molpharm.aspetjournals.org.
doi:10.1124/mol.108.046680.
Please see the related article on page 1444.

**ABBREVIATIONS:** ABC, ATP-binding cassette; Pgp, P-glycoprotein; MRP, multidrug-resistance-associated protein; BBB, blood-brain barrier; AED, antiepileptic drug; COX-2, cyclooxygenase-2.

Lazarowski et al. (2004b) noted an increase in Pgp expression, predominantly in brain capillary endothelium or neurons in rats experiencing repeated seizures for 4 or 7 days, respectively, suggesting that repeated seizures can trigger up-regulation of Pgp. Volk and Loscher (2005) extended these observations by electrically inducing seizures in rats, treating the rats with phenobarbital to determine responders and nonresponders to the drug, and subsequently examining Pgp expression in the brains of the two groups. They found up to a 4-fold increase of the Pgp-labeled area in capillary endothelial cells at the piriform cortex of nonresponders compared with responders (Volk and Loscher, 2005). These results were mirrored in studies of refractory epilepsy in humans. Increased expression of Pgp, as well as MRP, has been associated with refractory epilepsy in tuberous sclerosis (Lazarowski et al., 2004a). Increased levels of Pgp were found in astrocytes and blood vessel endothelium in hippocampal samples obtained from patients with refractory mesial temporal lobe epilepsy compared with control samples (Aronica et al., 2004). Ak et al. (2007) found increased expression of Pgp and MRP in neurons, astrocytes, and endothelial cells at the epileptogenic zone in samples taken from patients with focal cortical dysplasia (Ak et al., 2007). Increased levels of Pgp in the brain are clearly associated with seizure and are found in refractory epilepsy.

Animal models of drug-resistant epilepsy seem to firmly establish a role for Pgp as a target for increasing clinical response to current epilepsy treatments. Overexpression of Pgp has been demonstrated and the administration of a Pgp inhibitor such as tariquidar has been shown to overcome drug resistance in animal models of drug-resistant epilepsy (Volk and Loscher, 2005; Brandt et al., 2006; van Vliet et al., 2006). More recently, it was documented that adjuvant treatment with nimodipine, a calcium channel blocker that also inhibits Pgp activity, was able to restore the normal hippocampal pharmacokinetics of phenytoin with total seizure control, avoiding epilepticus status-associated death in a phenytoin-refractory epilepsy model (Höcht et al., 2007; Lazarowski et al., 2007b). There are anecdotal accounts of verapamil reversing drug-resistant epilepsy in humans (Summers et al., 2004; Iannetti et al., 2005). Two reports involving only single cases have appeared; overexpression of Pgp was not confirmed and inhibition of Pgp was not validated. As an aside, there remains the possibility that the success of Pgp inhibitors in controlling seizures in animal models, and possibly in humans, has mechanism other than inhibition of AED transport mediated by Pgp.

One major point of controversy centers around the identification of AEDs that are substrates for efflux by drug transporters, particularly by human Pgp. Although early studies seemed to suggest that some AEDs were transported by Pgp (Schinkel et al., 1996), recent studies by Baltes et al. (2007) and Crowe and Teoh (2006) imply that human and murine Pgp have different substrate specificities and that relatively few AEDs are transported by Pgp. However, the sensitivity of their model system was not determined. To validate the model system used, Madin-Darby canine kidney II or LLC-PK1 pig kidney cells transfected with MDR1 (Baltes et al., 2007) or Caco-2 cells (Crowe and Teoh, 2006), well known compounds that are readily transported by Pgp were used. What was not included was a compound that is a known substrate of Pgp but that has poor transport efficiency, thus enabling one to gauge the sensitivity of the assay. It may be that AEDs are Pgp substrates but are not so well transported that they can be detected by the model systems used. In addition, it is not clear whether Pgp levels in the model systems accurately represent Pgp levels in brain capillaries of patients with drug-resistant epilepsy.

Because significant transport of AEDs by human Pgp has not been conclusively demonstrated, investigators have recently asked whether other transporters including ABCG2 are involved. ABCG2 has been localized to the brain microvessel endothelium in rodents (Hori et al., 2004; Lee et al., 2007) and humans (Cooray et al., 2002) and has been found to colocalize with Pgp (Sisodiya et al., 2006), suggesting that it plays a critical role in the BBB. Recent studies do not seem to suggest that ABCG2 significantly transports many drugs used to treat epilepsy (Cerveny et al., 2006); however, definitive studies are needed.

In the current study by Bauer et al. (2008), we are provided with additional evidence supporting a role for Pgp in drug-resistant epilepsy. The authors demonstrate that Pgp overexpression can be induced locally by microinjection of glutamate, which increases levels of COX-2 (Bauer et al., 2007). Glutamate-injected rats were found to have significantly increased levels of Pgp in the right hilus, located ventral to the injection site, compared with vehicle-injected control rats (Bauer et al., 2007). Note that Bauer at al. (2008) also demonstrated that intracerebral microinjections of glutamate at nanomolar levels were sufficient to locally increase P-glycoprotein expression without seizure activity. The increased expression of Pgp was found to occur in the endothelium of existing capillaries and not newly formed capillaries. Pgp expression in more distal regions of the brain was not significantly affected. These particular data suggest that molecular factors inducing Pgp overexpression in the BBB, even in the absence of seizures, could precondition the refractory phenotype for some epileptic syndromes.

It should be noted that the focus of this very important finding was limited to BBB-vascular endothelial cells. In accordance with the recently defined "neurovascular unit" for stroke by del Zoppo (2006), we believe that the critical role for *ABCB1* gene induction should be extended to other brain cells, such as astrocytes and neurons, not only in hypoxic conditions (Lazarowski et al., 2007a) but also in epilepsy.

Several studies in cancer systems have posited a link between COX-2 expression and Pgp expression. Fantappiè et al. (2002). found that COX-2 and inducible nitric-oxide synthase expression was up-regulated in a doxorubicin-selected, Pgp overexpressing cell line. Similarly, Surowiak et al. (2005) found that invasive breast cancer tumor samples that were COX-2-positive also had higher expression of Pgp and that increased COX-2 and Pgp were negative prognostic factors. When Patel et al. (2002) transferred rat Cox-2 into rat mesangial cells via adenovirus, they noted an increase in Pgp expression and function that correlated with the increase in Cox-2 mRNA expression. The increase in Cox-2 and Pgp could be prevented by incubating infected cells with the Cox-2 inhibitor NS398, again reinforcing the link between COX-2 and Pgp (Patel et al., 2002). The results obtained by Bauer et al. (2008) continue this line of evidence, because Pgp up-regulation in the epileptic rat brain capillaries could be prevented by the COX-2 inhibitor celecoxib.

Thus, the findings of Bauer et al. (2008) suggest that

up-regulation of Pgp may be a local, but not global, effect and thus may not be able to be detected in studies that measure whole-brain uptake of drugs. Indeed, there is conflicting evidence with regard to uptake of Pgp substrates in the drug-resistant brain in humans. One study found no differential transport of verapamil in epileptogenic versus nonepiloeptogenic tissue in patients diagnosed with refractory epilepsy (Langer et al., 2007); another found that increased levels of Pgp were associated with decreased levels of a metabolite of oxcarbazepine (Marchi et al., 2005). Langer et al. (2007) point out that the data do not exclude the possibility that enhanced Pgp-mediated efflux in some regions of the brain is responsible for resistance to AEDs in some patients.

In oncology, there was a rush to clinical trial before the best inhibitors were developed and before it was clear in which cancers Pgp overexpression was important. This resulted in many negative trials and the failure to confirm a role for Pgp in multidrug resistance in cancer. In epilepsy, investigators have been cautious, awaiting clear and convincing evidence to confirm a role for Pgp (Sisodiya and Bates, 2006). Perhaps as a result of this careful approach, convincing animal data for a role in epilepsy have been gathered and are much stronger than any evidence attained for Pgp. The remaining question seems to be determining which substrates are involved. The article by Bauer et al. (2008) suggests a new strategy—COX-2 inhibitors for the prevention of drug resistance in epilepsy. Bauer et al. (2008) note that there is concern regarding the side effects of COX-2 inhibitors, which resulted in the removal of FDA approval for celecoxib (Sarkar et al., 2007), although the risk may be offset by the adverse impact of chronic refractory epilepsy. P-glycoprotein inhibitors have already been used in patients with chemotherapy (Peck et al., 2001; Pusztai et al., 2005; Kuppens et al., 2007) and some, such as tariquidar (Robey et al., 2004) and biricodar (Minderman et al., 2004), inhibit multiple transporters. Long-term use of these compounds has never been attempted because most cancer treatment schedules are episodic, and safety would be a concern. Despite the overall good health of Pgp-deficient mice, the increased toxicity with ivermectin suggests that there might be challenges in the event of xenobiotic exposure. These arguments aside, it may simply be time to translate the most promising animal studies to the clinic. A well designed clinical trial may allow a leap forward for the science of epilepsy and for patients suffering from the disease.



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