The Multidrug Transporter Hypothesis of Refractory Epilepsy: Corroboration and Contradiction in Equal Measure

Evaluation of the Role of P-Glycoprotein in the Uptake of Paroxetine, Clozapine, Phenytoin, and Carbamazapine by Bovine Retinal Endothelial Cells

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Expression of the drug transport proteins, including P-glycoprotein (Pgp), in the brain vascular endothelium represents a challenge for the effective delivery of drugs for the treatment of several central nervous system (CNS) disorders including depression, schizophrenia, and epilepsy. It has been hypothesized that Pgp plays a major role in drug efflux at the blood–brain barrier, and may be an underlying factor in the variable responses of patients to CNS drugs. However, the role of Pgp in the transport of many CNS drugs has not been directly demonstrated. To explore the role of Pgp in drug transport across an endothelial cell barrier derived from the central nervous system, the expression and activity of Pgp in bovine retinal endothelial cells (BRECs) and the effects of representative CNS drugs on Pgp activity were examined. Significant Pgp expression in BRECs was demonstrated by western analyses, and expression was increased by treatment of the cells with hydrocortisone. Intracellular accumulation of the well-characterized Pgp-substrate Taxol was markedly increased by the nonselective transporter inhibitor verapamil and the Pgp-selective antagonist PGP-4008, demonstrating that Pgp is active in these endothelial cells. In contrast, neither verapamil nor PGP-4008 affected the intracellular accumulation of [^{3}H]paroxetine, [^{14}C]phenytoin, [^{3}H]clozapine, or [^{14}C]carbamazapine, indicating that these drugs are not substrates for Pgp. Paroxetine, clozapine and phenytoin were shown to be Pgp inhibitors, while carbamazapine did not inhibit Pgp at any concentration tested. These results indicate that Pgp is not likely to modulate patient responses to these drugs.

Multidrug Resistance in Epilepsy: Rats with Drug-Resistant Seizures Exhibit Enhanced Brain Expression of P-Glycoprotein Compared with Rats with Drug-Responsive Seizures

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Medical intractability, that is, the absence of any response to antiepileptic drug (AED) therapy, is an unresolved problem in many patients with epilepsy. Mechanisms of intractability are not well understood, but may include alterations of pharmacological targets and poor penetration of AEDs into the brain because of increased expression of multiple drug-resistance proteins, such as P-glycoprotein (Pgp; ABCB1), capable of active brain extrusion of various drugs, including AEDs. Increased expression of Pgp has been reported in brain tissue of patients with refractory epilepsy, but there is a lack of adequate controls, that is, brain tissue from patients with drug-responsive epilepsy. In the present study, we used a rat model of temporal lobe epilepsy to examine whether AED responders differ from nonresponders in their expression of Pgp in the brain. In this model, spontaneous recurrent seizures develop after status epilepticus induced by prolonged electrical stimulation of the basolateral amygdala. The frequency of these seizures was recorded by continuous video-EEG monitoring before, during and after daily treatment with phenobarbital, which was given at maximum tolerated doses for 2 weeks. On the basis of their individual response to phenobarbital, rats were grouped into responders ($n = 7$) and nonresponders ($n = 4$). Pgp expression was studied

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by immunohistochemistry and showed striking overexpression in nonresponders compared with responders in limbic brain regions, including the hippocampus. The Pgp overexpression was confined to brain capillary endothelial cells that form the blood–brain barrier. The present data are the first to demonstrate that rats with drug-resistant spontaneous seizures differ from rats with drug-responsive seizures in their Pgp expression in the brain, thereby substantiating the multidrug transporter hypothesis of intractable epilepsy.

COMMENTARY

The last 15 years have witnessed the introduction of no fewer than 10 new antiepileptic drugs (AEDs) to the worldwide marketplace, representing an unprecedented advance in epilepsy therapeutics and a doubling in the number of effective treatments. Despite this welcome expansion of the pharmacological armamentarium, it is estimated that around 35% of people with epilepsy still do not respond adequately to drug therapy (1). Resistance to treatment for epilepsy occurs across a wide range of seizure types and to a variety of drugs, often with differing molecular mechanisms of action. Recently, considerable effort has focused on unraveling the complexities of treatment response for patients with epilepsy. Several theories have emerged that may explain, at least in part, the relatively poor prognosis for one-third of newly diagnosed epilepsy cases (2). Most of the attention has centered on the mechanisms by which AEDs penetrate the blood–brain barrier and how these mechanisms might be compromised in patients with refractory epilepsy (3).

Under normal physiological conditions, the integrity of the blood–brain barrier is maintained by the existence of tight junctions between endothelial cells, a lack of fenestrations, a relative paucity of pinocytotic vesicles, and by the expression of efflux transport proteins on the lumenal surface of capillary endothelial cells. These transport proteins are expressed throughout the body, predominantly in organs with excretory functions, and at blood–tissue barriers; they are believed to act as a physiologic defense mechanism, extruding xenobiotics from mammalian cells and affording protection of sensitive tissues. The most widely recognized of these transport systems are P-glycoprotein (Pgp) and the multidrug resistance-associated proteins (MRPs), which are members of the ATP-binding cassette superfamily. Pathologically elevated expression of Pgp has been implicated in contributing to the phenomenon of clinical drug resistance in a variety of conditions, including cancer, rheumatoid arthritis, and inflammatory bowel disease. Attention has turned to its possible role in drug-resistant epilepsy.

Accumulating evidence supports a localized overexpression of drug transport proteins in the region of experimentally induced seizure foci and in spatial association with a number of clinical neuropathologies, such as hippocampal sclerosis, cortical dysplasia, and dysembryoplastic neuroepithelial tumor, all of which are commonly associated with uncontrolled seizures (4). Anecdotal reports have linked overexpression of drug transport proteins with acute seizure activity, and a number of AEDs have been mooted as substrates for at least one of these transport systems (5,6). These experimental observations have spawned the multidrug transporter hypothesis, which proposes that refractory epilepsy may be the consequence of a localized overexpression of transport proteins that prevents antiepileptic agents from penetrating the blood–brain barrier in sufficient concentration—and would explain why patients are resistant to multiple AEDs with distinct mechanisms of action (7).

This contemporary theory is founded on three basic premises: (1) that overexpression of drug transporter proteins is exquisitely localized to the site of primary pathology, which prevents AED access to the seizure focus but allows drug penetration in other brain areas, as evidenced by the precipitation of CNS side effects in otherwise drug-resistant patients; (2) that overexpression of drug transporter proteins is exclusively observed in pharmacoresistant epilepsy patients and is not an epiphenomenon of the epileptic focus or underlying pathology; and (3) that the majority of (if not all) currently available AEDs are substrates for active efflux by one or more drug transporter proteins. Recent publications have addressed the latter two of these three principal issues.

The vast majority of studies that have demonstrated overexpression of drug transporter proteins have been performed on resected brain tissue from patients undergoing surgery for intractable epilepsy. These investigations are undermined by the lack of control tissue from drug-responsive subjects, who do not present for epilepsy surgery. Investigators have often resorted to utilizing adjacent, histologically normal tissue from epilepsy resections as control samples. Tissue obtained at autopsy or from nonepileptic patients undergoing other neurosurgical procedures have also been used. Whether this practice is sufficiently robust is debatable and, as a result, the question of whether overexpression of transporter proteins is exclusive to patients with pharmacoresistant seizure disorders remains to be resolved.

A recent study by Volk and Löscher has attempted to address the issue by investigating the expression of Pgp in a rat model of temporal lobe epilepsy in which the animals develop spontaneous recurrent seizures following a period of acute status

epilepticus. An evaluation of the efficacy of chronic phenobarbital in the spontaneous seizure phase permitted differentiation of the animals into responders and nonresponders on the basis of pre- and post-treatment seizure frequencies, recorded by continuous video-EEG monitoring. Subsequent immunohistochemical analysis revealed a significantly increased expression of Pgp in capillary endothelial cells of limbic brain regions in animals that were nonresponsive to phenobarbital. These findings suggest, for the first time, that animals with drug-resistant spontaneous seizures differ from their drug-responsive counterparts with respect to the expression of Pgp at the blood–brain barrier. This study would appear to corroborate the multidrug transporter hypothesis of refractory epilepsy and substantiate one of its principal caveats: that overexpression of a drug transporter protein is exclusive to nonresponders and not equally observed in drug-responsive subjects.

If multidrug transporters play a role in the distribution of AEDs across the blood–brain barrier and contribute to the phenomenon of drug resistance in epilepsy, then it is reasonable to assume that the majority of, if not all, AEDs are substrates for some form of active efflux that occurs at therapeutically relevant concentrations and is of sufficient capacity to obviate the elevation in serum levels associated with increased dose. A number of studies have attempted to address this important issue, with little or no consensus in their findings (5–7). In the absence of a gold-standard method for the identification of substrates, disparities are commonly, and perhaps conveniently, explained on the basis of the techniques selected for investigation. For some antiepileptic agents, such as carbamazepine, the available evidence is so contradictory that it is entirely inconclusive (8,9). In general, it would appear that AEDs could be classed, at best, as weak substrates for drug-transporter–mediated efflux.

A recent study by Maines and colleagues has added to the ongoing debate. These investigators employed bovine retinal endothelial cells in monolayer culture, as an in vitro model of the blood–brain barrier, in an effort to assess the mutual interaction between Pgp and a series of representative CNS drugs. Expression of Pgp in the model was confirmed by western blotting, and its functionality verified by the reversal of cellular paclitaxel accumulation in the presence of known Pgp inhibitors. Interestingly, a parallel model employing rat brain endothelial cells was devoid of function, despite the expression of Pgp on the cell surface. In summary, none of the compounds under investigation, including phenytoin and carbamazepine, appeared to be a substrate for Pgp-mediated efflux. A weak inhibition of Pgp was observed with phenytoin, but only at supra-therapeutic concentrations. At face value, these findings would appear to question the multidrug transporter hypothesis and potentially contradict one of its central tenets—that first-line AEDs are substrates for drug-transporter–mediated efflux.

As with all such laboratory investigations, these publications should be considered with a degree of caution. If nothing else, extrapolating directly from either rodent- or bovine-based experimental paradigms to humans is questionable. The compelling findings of Volk and Löscher are further tempered by a lack of information regarding the functionality of overexpressed Pgp and the fact that overexpression, itself, was only confirmed some months after the period of phenobarbital treatment. While these limitations are acknowledged to be a direct result of a necessarily strict experimental design and are not intended as criticisms, further work is clearly required if the wide-ranging implications of the study are to be validated. The observations of Maines and colleagues are similarly inconclusive, being both limited in scope and potentially deceptive.

Pgp may be the prototype for the multidrug transporter hypothesis, but the hypothesis is not solely dependent on Pgp or its ability to transport AEDs. A vast array of drug transporter proteins, including the MRPs and major vault protein have been shown to be upregulated in the vicinity of the epileptic focus (10,11). It is reasonable to speculate that while phenytoin and carbamazepine might not be subject to Pgp-mediated transport, they are potential substrates for some other efflux system. Again, further studies are required to clarify the transport profile of AEDs.

There is little doubt that the role of transporter proteins in the response to the drug treatment of epilepsy is an important contemporary issue. However, the three central prerequisites of the multidrug transporter hypothesis have, thus far, not been met and questions of clinical significance remain. The biological plausibility of the hypothesis appears to lend credence where it is not yet deserved, and the preponderance of positive findings as well as the often casual dismissal of contradictory data suggest a tendency for research in this area to follow a perceived wisdom. The provenance of the multidrug transporter hypothesis will only be confirmed once all AEDs are identified as substrates for some form of active efflux and human studies verify that localized overexpression is a process that is exclusive to nonresponsive patients. Even then, the most ardent advocates of the hypothesis acknowledge that, at best, drug transporters are likely to constitute just one of many contributory mechanisms to the phenomenon of pharmacoresistance in epilepsy. The work of Volk and Löscher is persuasive, but not unequivocal. The study by Maines and colleagues is damning, but not excessively so. For the moment at least, the hypothesis remains unproven.

by Graeme J. Sills, PhD

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