

ANTIEPILEPTIC DRUG TRANSPORT—OF MICE AND MEN

Differences in the Transport of the Antiepileptic Drugs Phenytoin, Levetiracetam and Carbamazepine by Human and Mouse P-Glycoprotein. Baltes S, Gastens AM, Fedrowitz M, Potschka H, Kaever V, Loscher W. *Neuropharmacology* 2007;52(2):333–346. In view of the important role of P-glycoprotein (P-gp) and other drug efflux transporters for drug distribution and resistance, the identification of compounds as substrates of P-gp-mediated transport is one of the key issues in drug discovery and development, particularly for compounds acting on the central nervous system. In vitro transport assays with P-gp-transfected kidney cell lines are widely used to evaluate the potential of compounds to act as P-gp substrates or inhibitors. Furthermore, such cell lines are also frequently utilized as a substitute for more labor-intensive in vitro or in vivo models of the blood–brain barrier (BBB). Overexpression of P-gp or members of the multidrug resistance protein (MRP) family at the BBB has been implicated in the mechanisms underlying resistance to antiepileptic drugs (AEDs) in patients with epilepsy. Therefore, it is important to know which AEDs are substrates for P-gp or MRPs. In the present study, we used monolayers of polarized MDCKII dog kidney or LLC-PK1 pig kidney cells transfected with cDNA containing either human MDR1, MRP2 or mouse *mdr1a* and *mdr1b* sequences to measure the directional transport of AEDs. Cyclosporin A (CsA) and vinblastine were used as reference standards for P-gp and MRP2, respectively. The AEDs phenytoin and levetiracetam were directionally transported by mouse but not human P-gp, whereas CsA was transported by both types of P-gp. Carbamazepine was not transported by any type of P-gp and did not inhibit the transport of CsA. In contrast to vinblastine, none of the AEDs was transported by MRP2 in transfected kidney cells. The data indicate that substrate recognition or transport efficacy by P-gp differs between human and mouse for certain AEDs. Such species differences, which are certainly not restricted to human and mouse, may explain, at least in part, the controversial data that have been previously reported for AED transport by P-gp in preparations from different species. However, because transport efficacy of efflux transporters such as P-gp or MRP2 may not only differ between species but also between tissues, the present data do not exclude that the AEDs examined are weak substrates of P-gp or MRP2 at the human BBB.

Valproic Acid Is Not a Substrate for P-Glycoprotein or Multidrug Resistance Proteins 1 and 2 in a Number of In Vitro and In Vivo Transport Assays. Baltes S, Fedrowitz M, Tortos CL, Potschka H, Loscher W. *J Pharmacol Exp Ther* 2007;320(1):331–343. The antiepileptic drug valproic acid (VPA) is widely used in the treatment of epilepsy, bipolar disorders, and migraine. However, rather high doses are required for the clinical effects of VPA, which is due to its relatively inefficient delivery to the brain. The poor brain distribution of VPA is thought to reflect an asymmetric transport system at the blood–brain barrier (BBB). Based on recent data from in vitro experiments, multidrug resistance proteins (MRPs) have been proposed to be involved in the efflux transport of VPA at the BBB. In the present study, we used different experimental in vitro and in vivo strategies to evaluate whether VPA is a substrate for MRPs or the efflux transporter P-glycoprotein (P-gp). In contrast to known P-gp or MRP substrates, such as cyclosporin A or vinblastine, no directional transport of VPA was observed in cell monolayer efflux assays using the kidney cell lines Madin Darby canine kidney II and LLC-PK1, which had been transfected with either human or mouse cDNAs for the genes encoding P-gp, MRP1, or MRP2. Likewise, no indication for efflux transport of VPA was obtained in a rat microdialysis model, using inhibitors of either P-gp or MRPs. Furthermore, a significant role of MRP2 in brain efflux of VPA was excluded by using MRP2-deficient rats. Our data do not support the hypothesis that MRP1 or MRP2 is involved in the efflux of VPA from the brain. Thus, the molecular identity of the putative transporter(s) mediating the active efflux of VPA from the brain remains to be elucidated.

COMMENTARY

The drug transporter hypothesis of pharmacoresistant epilepsy has gained considerable favor in recent years. In its simplest form, it states that a localized overexpression of drug transporter proteins, such as P-glycoprotein (P-gp) and multidrug resistance associated proteins (MRPs), in the region of the epileptic focus, can reduce the efficacy of antiepileptic drugs (AEDs) by limiting their ability to penetrate the blood–brain barrier (1). This theory offers a straightforward and biologically plausible explanation for the failure of multiple AEDs,

often with differing mechanisms of action, to control seizures in a significant proportion of patients with localization-related epilepsies. It is, however, based on the fundamental assumption that the majority of (if not all) currently available AEDs are substrates for active efflux by one or more drug transport systems.

The transport of AEDs by P-gp and related proteins has been the subject of much investigation and mounting confusion. Initial in vitro studies, employing a neuroectodermal cell line and transfected porcine kidney epithelial cells, suggested that phenytoin was subject to P-gp-mediated efflux (2,3), albeit to a greater extent in cells expressing the rodent form of the protein than its human equivalent. Investigations using genetic knockout mice that are reportedly devoid of functional

P-gp at the blood–brain barrier failed to demonstrate enhanced penetration of radiolabeled phenytoin in the brain of *mdr1a* knockout mice (which most likely is due to the confounding influence of similarly radiolabeled metabolites) but did reveal an intriguing regional selectivity in phenytoin transport, with accumulation in hippocampus but not cerebellum, of the *mdr1a/1b* double knockout (3,4). A further pharmacokinetic study employing *mdr1a* knockouts concluded that only topiramate, of seven common AEDs under examination, was potentially susceptible to P-gp-mediated transport (5). Finally, a detailed investigation using multiple experimental techniques, including *mdr1a* knockouts, Caco-2 cell monolayers, and rhodamine-123 efflux from human lymphocytes, failed to provide any indication that carbamazepine was a substrate for P-gp (6). Thus, the evidence that commonly used AEDs are substrates for transporter-mediated efflux is modest, at best.

These individual studies were somewhat overshadowed, however, by a series of elegant investigations in experimental animals that employed bilateral intracerebral microdialysis and appeared to suggest that several AEDs, including phenobarbital, phenytoin, carbamazepine, lamotrigine, felbamate, and oxcarbazepine (but interestingly not levetiracetam), are transported to some extent by P-gp and/or MRPs (7–9). Such was the clarity and, some might argue, convenience of this evidence that the promiscuity of efflux transporters in terms of their ability to extrude AEDs became accepted wisdom, almost overnight. Any concern regarding species differences or the capacity of these systems to transport AEDs at clinically relevant concentrations was ignored in the wave of optimism arising from the identification of a credible mechanism of drug resistance in epilepsy.

Fortunately, a degree of circumspection has returned to this area of epilepsy research. Two recent publications highlight the difficulty in extrapolating experimental findings between species as well as in relying on a single model or experimental technique to characterize a complex pharmacological relationship. These papers come from the same research group that published the microdialysis data described above but have now turned their attention to more molecular matters. The results make interesting reading, particularly in light of previous findings and the support those findings provided for the drug transporter hypothesis.

The manuscripts, reviewed here, describe a series of in vitro studies examining the interaction between commonly used AEDs and drug transporter proteins, using porcine and canine kidney epithelial cells transfected with mouse and human forms of P-gp and MRPs. In this paradigm, phenytoin and levetiracetam displayed unidirectional transport characteristics of P-gp substrates in cells transfected with the mouse form of the protein but not the human equivalent. Carbamazepine and valproic acid were not transported by cells expressing P-gp of either species,

and none of the four AEDs examined in these two research articles was subject to MRP-mediated efflux. When considered alongside the promiscuity of AED transport described in the in vivo rat microdialysis model, these findings are much more conservative in their implications. They not only emphasize a hitherto under appreciated species distinction in the substrate profile of drug transporters (most strikingly exemplified by the apparent efflux of levetiracetam by mouse but not rat P-gp), but perhaps more importantly, they offer no evidence to suggest that any AED is a substrate for any of the major human efflux transporters.

Other recent in vitro studies have been similarly negative, although none have compared the substrate profile of rodent and human drug transporters in a single investigation or afforded a direct contrast with in vivo findings. In 2003, Weiss et al. reported that several AEDs including carbamazepine, phenytoin, lamotrigine, and valproate could interact with human P-gp expressed in porcine kidney epithelial cells but only at concentrations above the upper limit of the therapeutic range (10). Neither phenytoin nor carbamazepine appeared to be a substrate for P-gp-mediated efflux in an in vitro model of the blood–brain barrier employing bovine retinal endothelial cells in monolayer culture (11); and, there was similarly no evident transport of phenobarbital, carbamazepine, vigabatrin, lamotrigine or gabapentin in a subclone of the Caco-2 cell line that demonstrates extensive P-gp expression (12). Interestingly, phenytoin was transported to a modest extent in these cells, but the effect was not reversible in the presence of known P-gp inhibitors, suggesting a lack of selectivity.

This clear disparity in the findings of experimental studies designed to assess the interaction between drug transporter proteins and AEDs is perplexing. With the exception of phenytoin transport by rodent P-gp, in vitro studies are largely negative, while data derived from knockout mice are inconclusive (possibly a result of functional redundancy in transport systems and the upregulation of alternative proteins to replace those that have been deactivated), and levetiracetam aside, in vivo microdialysis results are overwhelmingly positive. Despite considerable research efforts, the fundamental question of whether AEDs are substrates for transporter-mediated efflux remains. The authors of the two manuscripts discussed here quite rightly propose that their in vitro work does not exclude the possibility that antiepileptic agents might be transported to some extent by efflux transporters, but the balance of opinion has shifted in light of their new data, and particularly in relation to the inter-species differences in substrate specificity. While previously AEDs were afforded the benefit of the doubt in order to satisfy a biologically plausible hypothesis, it may now be more appropriate to suggest that, under normal circumstances and until proven otherwise, they are not substrates for human transporter-mediated efflux.

The credence of the drug transporter hypothesis of refractory epilepsy has been diminished by these latest findings, but it may be a little premature to declare it irreparably undermined. There are still many aspects of this theory that require investigation. If the characteristics of human blood–brain barrier transporters are sufficiently distinct from those expressed in other tissues or if the expression and functionality of drug transporter proteins is altered in disease states and/or influenced by genetic factors, then these still represent avenues for potential clinical exploitation. Models and experimental systems that adequately mirror the appropriate physiological and pathological circumstances are required, and there are now clear grounds for a detailed examination of the individual properties of human and rodent efflux transporters and an evaluation of whether any observed disparity might offer further therapeutic advantage. It is possible that the drug transporter hypothesis of refractory epilepsy is one that will never be entirely provable or disprovable. If nothing else, however, it is likely to keep investigators busy for a number of years to come.

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