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Variability in expression of the human MDR1 drug efflux transporter and genetic variation of the *ABCB1* gene: implications for drug-resistant epilepsy

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Abstract

Among individuals diagnosed with epilepsy, as many as one in three develop resistance to antiepileptic drugs (AEDs) thus rendering their seizures refractory to treatment. Despite current antiepileptic drugs (AEDs) having a variety of modes of action, seizures in drug-resistant individuals often persist even after treatment with two or more drugs. The underlying cause of this broad resistance is currently under debate, but two dominant theories have emerged and have been widely studied. Here we discuss current literature investigating the “transporter theory”, the idea that individuals present with drug resistance due to genetic variability in the *ABCB1* gene encoding the efflux transporter multidrug resistance protein 1 (MDR1). Results of *in vitro* and *in vivo* studies suggest that variability in the expression of the MDR1 transporter may be closely tied to drug resistance. While there is much support for this hypothesis from molecular and mechanistic studies, population-based studies of *ABCB1* polymorphisms are divergent in their conclusions, and there is need for additional investigations.

Keywords

epilepsy; antiepileptic drugs (AEDs); ATP binding cassette membrane protein 1 gene (*ABCB1*); drug resistant epilepsy; P-glycoprotein (P-gp); multidrug resistance protein 1 (MDR1)

1. Introduction

Despite advances in treatment and therapy of epilepsy, a large subset of those individuals affected by this prevalent disorder remain susceptible to seizures despite treatment. Drug-

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resistant epilepsy is a specific condition that affects as many as a third of epileptic patients. Frequently, patients diagnosed with epilepsy prove to be resistant to several independent drug treatments, despite these medications having divergent modes of action (MOAs). The International League Against Epilepsy (ILAE) recently consolidated a clinical diagnosis of the condition, defining it as a patient being diagnosed with an epileptic condition and having subsequently failed no fewer than two trials of clinically-approved antiepileptic drugs (AEDs). A plan of treatment for patients diagnosed with drug-resistant epilepsy was also outlined [1]. The underlying cause of resistance to multiple anti-convulsant drug treatments remains a topic of current debate, though two major theories have gained support in the field. The two predominant theories currently supported and under investigation are the “transporter theory”, which will be further discussed, and the “target theory”, which posits that AED targets themselves either develop or have intrinsic loss of sensitivity to the medications in resistant individuals.

The target theory has been amply considered in a number of recent reviews. Briefly, the target theory suggests that resistance to AEDs can occur due to the selective acquisition of mutations or altered regulation of the molecular targets of AEDs themselves. When a mutation occurs in a specific target, many or all drugs that act upon that target may become ineffective. Thus, under the target theory, a change that affects a specific target (for instance a voltage-gated sodium channel or the GABA_A receptor) can result in class-wide resistance to drugs that share the target. In addition, some AEDs have complex modes of action whereby they act upon more than one cellular target, leading to complexities in the breadth of drugs that are impacted by resistance.

This review will focus primarily on the current state of affairs of research designed to investigate the transporter theory, as there is strong evidence that the acquisition of drug efflux transporter dysfunction can lead to epilepsy that is refractory to treatment with multiple AEDs. The transporter theory seems especially attractive as a hypothesis to account for the occurrence of simultaneous resistance to a wide spectrum of AEDs, often with entirely distinct MOAs. The transporter theory proposes that an overexpression of drug efflux transporters at the cellular level alters pharmacokinetics sufficiently to prevent a prescribed medication from reaching an effective intracellular concentration, or having insufficient intracellular residency time to act upon its target. This theory is attractive because it can readily account for the ineffectiveness of multiple drugs despite dissimilar MOAs. The multi-drug resistance notion leads to the theory that patient-specific differences in the expression of drug efflux transporters are a major cause of inter-individual efficacy of AEDs. Indeed, variability in the density of these transporters has been shown in numerous studies to result in altered responses to medications in a wide array of conditions and relevant examples will be outlined in more detail in this review.

The efflux transporters with the best evidence to implicate them as players in drug-resistant epilepsy are encoded by the ATP Binding Cassette membrane protein (*ABC*) genes. Specifically, *ABCB1* (member 1 of subfamily B of the ABC transporters) encodes the drug efflux transporter multidrug resistance protein 1 (MDR1, also known as P-glycoprotein, P-gp). Mechanistic and molecular studies implicate MDR1 as being especially involved in the acquisition of drug-resistant epilepsy. A number of genome-wide association studies have

also provided evidence to support the notion that genomic variability may alter the expression or function of the MDR1 transporter and thus impact the occurrence of multi-drug resistant phenotypes among epilepsy patients. However, conflicting population-based studies have no association or complex genetic effects upon AED resistance, and the cases for and against human genetic variation in AED resistance are discussed later in this review.

2. Current anti-epileptic drugs

Epilepsy is a prevalent neurological disorder marked by abnormal activity in the brain which causes spontaneous and recurrent seizure activity. Over the past two decades a total of 23 medications have been approved by the U.S. Food and Drug Administration (FDA) for treatment of such seizure activity. Antiepileptic drugs exhibit a variety of MOAs, but in general they are designed to diminish neuronal excitability. Reduced excitability can be achieved by modulating the activity of several relevant drug targets, and resistance has been described in the literature for a large number of AEDs with differing MOAs (Table 1). A number of the drugs listed in Table 1 inhibit voltage-gated sodium channels, and some of these drugs have a secondary MOA whereby they increase the activity of GABA_A receptors. Other AEDs primarily affect GABA synthesis or GABA_A receptors without impacting sodium channel function. Yet other AEDs target synaptic vesicle glycoprotein 2A (SV2A), which allows these drugs to modulate neurotransmitter release into synapses. Finally, several approved AEDs target other receptors, or have poorly understood MOAs. Despite this wide array of drugs with a variety of MOAs, every category of currently approved AEDs can become ineffective in many patients due to the acquisition of drug resistance (Table 1). Because resistance to multiple AEDs frequently develops concurrently, it is likely that broad spectrum resistance has little to do with the individual MOAs of specific drugs. For this reason, the transporter theory is especially attractive.

3. MDR1

MDR1 is one of the most extensively studied transporters in the ATP binding cassette (ABC) super family of transport proteins. The MDR1 transporter protein is encoded by the *ABCB1* gene in humans and the genes *Mdr1a/1b* in mice. MDR1 is an ATP-dependent efflux transporter (Fig. 1) and has been implicated in a number of conditions and pharmacological effects involving drug efflux. The action of MDR1 can render drugs ineffective via its action, which leads to drug transport out of target cells. A second transporter, MDR2, encoded by the *ABCB4* gene also exists. These transporters are widely expressed across organs and is present especially in tissues that are active in compound transport, such as kidney tubules, columnar intestinal cells, and endothelial cells in brain capillaries, which form the blood brain barrier (BBB). This anatomical distribution combined with the efflux function of these transporters underlies their significant role in the removal and excretion of toxins and xenobiotic substances [2].

It is well established that overexpression of the efflux membrane transporter MDR1 can result in reduced efficacy of medications in a wide array of diseases and medications [3, 4, 5]. High densities of MDR1 transporters are able to expel medications from the cell at rates that effectively reduce the concentration of the drug in question at its intracellular target and

thus severely reduces the efficacy of the drug (Fig. 1). Both gain- and loss-of-function studies have been performed, which further support the inverse correlation between expression of MDR1 transporter and efficacy of medications.

Upregulation of MDR1 is consistently associated with reduced drug efficacy [3, 4, 5]. One study shows that upregulation of the MDR1 transporter in cultured proximal renal tubule cells yields reduced toxicity due to cadmium exposure. As expected, the addition of MDR1 inhibitors restores cadmium toxicity to levels similar to those of naive cells that have not undergone MDR1 upregulation [3]. A subsequent study indicates that upregulation of MDR1 in rodents at the BBB increased transport efficiency in the capillaries. By increasing the expression of MDR1 *in vivo*, then isolating the brain capillaries and bathing them in solutions containing [N- ϵ (4-nitrobenzofurazan-7-yl)-D-Lys⁸]-cyclosporine A (NBD-CSA), luminal accumulation of the fluorescent cyclosporine A derivative, NBD-CSA, in the capillaries was significantly increased in the transporter-upregulated tissue as compared to control samples [4]. Of particular relevance for epilepsy, it has been shown that seizure activity *in vivo* can upregulate expression of MDR1 due to elevated levels of glutamate [5]. A related study determined that increased expression of MDR1 in rodent epilepsy models negatively correlates with retention of phenytoin in the brain, thus suggesting that an overexpression of this transporter is sufficient to reduce retention of exogenous compounds (such as AEDs) in target cells [6]. Together, these observations provide evidence for the potential involvement of MDR1 in export of exogenous compounds from neural endothelial cells such as those of the BBB. However, many patients exhibit multi-drug resistance even at very early stages after onset of seizure activity. Therefore, it is likely that many cases of multidrug resistance can be the result of intrinsic variability in endogenous patient-specific transporter expression levels.

Since there is extensive evidence that the overexpression of MDR1 adversely inhibits intracellular drug accumulation, the inhibition of this transporter may provide a means to increase drug effectiveness. Indeed, inhibition of MDR1 by the inhibitor tariquidar established control of seizures in nearly all epileptic rats co-administered with the anti-seizure compound phenytoin [7]. Similar results have been found in other organ systems, such as the liver, where it was found that knock out of the related transporter MDR2 (encoded by the rodent *Abcb4* gene) results in greater rates of liver dysfunction when *Abcb4*^{-/-} knockout mice were administered a hepatotoxic diet rich in cholate [8].

Several animal experiments demonstrate that knockout of *Abcb1* results in an increase in drug absorption and activity. In two independent studies focusing on neurologically-active drugs, knockout of *Abcb1* resulted in the increased accumulation of olanzapine and methadone across the BBB [9, 10]. A similar study however found that while genetic knockout of *Abcb1* did increase concentrations of the antidepressants doxepin, venlafaxine, and paroxetine, concentrations of mirtazapine remained insignificantly altered in comparisons of knockout and control animals [11]. While these results in general suggest that genetic variations in *Abcb1* (*ABCB1* in humans) can broadly impact drug delivery to the brain, not all drugs that can cross the BBB are affected by MDR1-mediated efflux. Further support for the potential of MDR1 expression as a candidate for drug resistance has been provided by an extensive number of studies [12, 13, 14, 15, 16].

4. Genetic variations of *ABCB1* and their role in drug transport and resistance

The animal studies described above establish a defined role for MDR1 in drug resistance. Based on these foundational studies, intensive research has been focused on the importance of human genetic variation in the gene encoding the MDR1 protein, *ABCB1*, and possible effects of genetic variants in and near *ABCB1* upon drug efficacy and adverse events. There are several common single nucleotide polymorphisms (SNPs) in the genomic region that encompasses the human *ABCB1* gene and some of the most common polymorphisms are rs1128503 (C1236T), rs2032582 (G2677T), and rs1045642 (C3435T). Of these, rs1045642 (C3435T) has been implicated in reduced antiepileptic drug efficacy. More generally, *ABCB1* polymorphisms are strongly associated with variable drug function in an array of medications used for a range of diseases. For example, individuals carrying a homozygous haplotype for rs1128503T-rs2032582T-rs1045642T have an approximately 5-fold chance of requiring a higher methadone dose than individuals who carry a heterozygous haplotype for these SNPs, with implications for the successful treatment of heroin dependence [17]. In the treatment of acute coronary syndromes by administration of ticagrelor or clopidogrel, individuals with a genotype conferring increased expression of MDR1 had significantly higher rates of cardiovascular death, myocardial infarction, and stent thrombosis [18]. These observations are in line with the transporter theory and suggest that drug transporter substrate specificity is a significant contributor to mechanisms that confer resistance to drugs, regardless of the specifics of their MOAs. Of particular interest, the rs1045642 (C3435T) polymorphism is associated with reduced response to clopidogrel and prasugrel in treatment of cardiovascular disease. Occurrence of this SNP genotype significantly associated with the incidence of cardiovascular death, myocardial infarction, and stroke [19]. Though these studies do not investigate drug resistant epilepsy itself, they do lend support for the notion that MDR1 could also be involved in AED drug resistance.

5. Clinical evidence of *ABCB1* genetic polymorphisms and associated AED resistance

While the involvement of MDR1 (and to a lesser extent MDR2) in the acquisition of drug resistance has broad support, a definitive for MDR1 in multi-drug resistance to AEDs is only now emerging. Several studies have shown a positive correlation between overexpression of MDR1 and the occurrence of resistance to AEDs in epileptic patients. In one study, it was found that several genes encoding drug efflux transporters (including MDR1 and MDR2 and others) are over-expressed in BBB endothelial cells from epilepsy patients whose seizures were refractory to treatment [20]. In related studies, the expression of MDR1 was investigated in surgical samples and brain lesion material, and MDR1 and related P-glycoproteins were found to be highly expressed in BBB endothelial cells and also in neurons and astrocytes, suggesting that drug efflux transporter function in both neural endothelial and non-endothelial cells (neurons and glia) are of importance [21, 22, 23]. Another study measured the brain-to-plasma ratios of metabolites of oxcarbazepine, an anti-seizure medication in brain resection samples from treatment-resistant epilepsy patients and control samples [24]. This study found that intracellular levels of 10-OHCBZ, a metabolite

of oxcarbazepine, are inversely correlated with MDR1 mRNA expression levels, and that intracellular levels of 10-OHCBZ are increased when a P-gp inhibitor is added. In another functional study, an *in vitro* cell co-culture model of the human BBB was developed using endothelial cells obtained from human neurovasculature and human astrocytes. The cells were obtained from tissue samples from individuals with drug-resistant epilepsy and control subjects whose seizures were pharmacologically alleviated. In these co-cultures, phenytoin transport was 10-fold less when epithelial cells from drug-resistant subjects were used, and this was rescued by inhibition of MDR1 [25].

The evidence to suggest that MDR1 and related transporters play a significant role in many cases of drug-resistant epilepsy is strong based on the molecular and mechanistic studies presented in the above paragraph. However, the picture is less clear when examined from the perspectives of human genetic variation and pharmacogenomics. A complex clinical study of North Indian patients which included 392 epilepsy patients who were treated with a variety of AEDs, identified a set of responding and non-responding patients, however, there was no correlation between the presence of *ABCB1* SNPs and failure to respond to AED treatment [26]. Another study evaluated the potential association of *ABCB1* sequence polymorphisms in patients whose intestinal density of MDR1 had been assessed found a correlation between genetic variants and drug retention. Of the 29 exons contained in the *ABCB1* gene, the 26th exon features a polymorphism at position 3435 that is strongly related to variability in MDR1 expression, rs1045642 (C3435T). A study by Hoffmeyer et al found that individuals who were homozygous for rs1045642 T/T had significantly lower levels of MDR1 in the intestine, and these individuals had elevated plasma levels of orally administered digoxin and those with a homozygous rs1045642 C/C genotype had comparatively higher levels of MDR1 expression [27]. The Hoffmeyer et al study was the first to suggest an association between this SNP and potential multi-drug resistance, but this analysis assessed only 21 individuals. Since then, several additional studies have attempted to corroborate this finding, some investigating a potential role for this polymorphism specifically in drug resistant epilepsy. Three recent studies have supported the notion that the rs1045642 (C3435T) SNP is associated with drug resistance in epilepsy, and these studies assessed hundreds of human subjects [28, 29, 30]. However, other studies of genetic variation in and near *ABCB1* failed to find significant associations between *ABCB1* SNPs and drug-resistant epilepsy in Caucasian and Korean populations [31, 32]. In another study of Chinese Han individuals, the *ABCB1* C3435T allele was significantly associated with drug-resistant epilepsy [33]. In another study of small sample size, expression levels of *ABCB1* mRNA were not correlated with the C3435T SNP [34]. A study of Japanese subjects found that while there was an association between the rs1045642 (C3435T) polymorphism and drug resistance, these individuals were more likely to have the TT homozygous genotype [35]. To further complicate the matter, though most AEDs are known substrates for MDR1-mediated transport, it has been determined that some AEDs are unaffected by MDR1 expression levels, suggesting that they are not substrates of this transporter [36]. This observation suggests that, if MDR1 expression is indeed a factor involved in some cases of AED drug resistance, variability in MDR1 density may not be causal in all cases for all AED drugs. In short, there is at present conflicting information in the literature about the significance of specific *ABCB1* polymorphisms and their potential role in conferring AED-resistance

phenotypes and there is a need for additional studies to clarify the matter of whether or not genetic variants exist that can predispose certain epilepsy patients to resistance to AED drugs. It is possible that non-heritable mechanisms can lead to increases in MDR1 expression, including gene amplification, or modifier loci that are unique to certain ethnicities, and these possibilities warrant further study.

6. Conclusions

Overall, the acquisition of resistance to AEDs can occur by a variety of mechanisms. It is clear that many cases of resistance to a single AED are due to acquired changes to specific drug targets themselves (whether mutational or regulatory in nature). Also, multiple AEDs can share the same target, and it is therefore not surprising that drug-resistant epilepsy can occur in a class-wise fashion to all drugs that share a common target. However, many cases of drug-resistant epilepsy occur in which simultaneous resistance occurs to a broad spectrum of drugs with dissimilar MOAs and targets. For these, the transporter hypothesis is especially attractive. As demonstrated by intensive research over the past two decades, a good case exists for the involvement of elevated MDR1 expression as having a role for drug resistant epilepsy. This view is supported by experimental models using animals and in cell culture, but investigations into human polymorphisms at *ABCB1* have yielded divergent results. Though several studies have found an association between *ABCB1* polymorphisms and the development of resistance to AEDs, this effect may be a feature of only certain ethnic populations [33, 35]. In addition, because resistance to AEDs is often an acquired trait, it is likely that the mechanisms responsible for acquired resistance occur in somatic cells (neurons) and therefore acquired resistance is not likely to be heritable. Furthermore, it is likely that there are other factors that play a role in the development of resistance to AEDs, and not all AEDs subject to resistance are substrates of MDR1 [36]. Despite the lingering debate surrounding AED drug resistance, there is a large set of published reports that suggest that increased MDR1 expression may reduce drug efficacy of several AEDs. Potential research directions include additional studies that attempt to break AED resistance through the use of MDR1 inhibitors (Fig. 1), a strategy that is supported at present by basic research finding in animals and cell culture.

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Abbreviations

<i>ABCB1</i>	ATP binding cassette membrane protein 1 gene
AED	antiepileptic drug
BBB	blood brain barrier
MDR1	multidrug resistance protein 1
MOA	mode of action

P-gp	p-glycoprotein
SNPs	single nucleotide polymorphisms

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- of special interest
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Figure 1. Mechanism of MDR1 transporter function in the blood brain barrier (BBB) and its proposed role in drug resistance. **A.** MDR1 transporter plays a role in efflux of foreign substances and toxins. The ATP driven transporter pump drugs from the intracellular space of endothelial cells back into peripheral blood. **B.** Overexpression of MDR1 is a proposed mechanism underlying multidrug resistance. Over-abundance of MDR1 transporters can diminish concentrations of drugs within target cells, by decreasing their ability to cross the BBB to the cerebral spinal fluid (CSF). **C.** Proposed model for the reversal of drug resistance by use of MDR1 inhibitors to decrease efflux of drugs out of vascular endothelial cells that participate in BBB function.

Table 1.

AEDs approved for treatment of seizure activity, their modes of action, and literature documenting clinical instances of failure of these medications in treatment of epileptic patients. Selected references of relevance to the acquisition of drug resistance are presented.

Medication	Approved	Mode of Action	Resistance Cited in Clinical Literature
Briviact (brivaracetam)	2016	Selective affinity for synaptic vesicle protein 2A (SV2A), exact method unknown	37, 38, 39
Carnexiv (carbamazepine)	2016	Thought to block sodium channels, exact method unknown	40, 63
Qudexy XR (topiramate)	2014	Blocks voltage gated sodium channels, increases activity of gamma-aminobutyrate at some GABA-A receptor subtypes, antagonist of AMPA/kainite glutamate receptor subtype, inhibits carbonic anhydrase enzyme	40, 41
Aptiom (eslicarbazepine acetate)	2013	Blocks voltage gated sodium channels	42, 43, 44
Trokendi XR (topiramate)	2013	Blocks voltage gated sodium channels, increases activity of gamma-aminobutyrate at some GABA-A receptor subtypes, antagonizes AMPA/kainite glutamate receptor, inhibits carbonic anhydrase enzyme	40, 41
Fycompa (perampanel)	2012	AMPA-type glutamate receptor antagonist	45, 46, 47
Oxtellar XR (oxcarbazepine, extended release)	2012	Blocks voltage gated sodium channels	24, 40, 48
Potiga (ezogabine)	2011	Activator of KCNQ ion channels	49
Sabril (vigabatrin)	2009	Irreversible enzyme activated GABA transaminase inhibitor	40, 50
Banzel (rufinamide)	2008	Thought to prolong inactivation state of sodium channels	51, 52
Vimpat (lacosamide)	2008	Increases slow inactivation of sodium channels, interacts with collapsing response mediator protein 2 (CRMP-2)	53, 54
Lyrica (Pregabalin)	2004	Modulates voltage gated calcium channels	40, 55
Neurontin (gabapentin)	2000	Method unknown	40, 56, 57
Trileptal (oxcarbazepine)	2000	Blocks voltage gated sodium channels	40, 48
Zonegran (zonisamide)	2000	Thought to block sodium channels and reduces voltage dependent, transient inward currents (T-type calcium currents)	58, 59
Kepra (levetiracetam)	1999	Regulates neurotransmitter release by binding to synaptic vesicle protein (SV2A) receptors	40, 60
Lamictal (lamotrigine)	1999	Thought to inhibit voltage gated sodium channels, exact method unknown	40, 61, 62
Topamax (topiramate)	1999	Thought to block voltage gated sodium/calcium channels, augment GABA-A activity at the receptor level, and antagonize AMPA/kainite glutamate receptors	40, 41
Lamictal Chewable Dispersible Tablets (lamotrigine)	1998	Thought to inhibit voltage gated sodium channels, exact method unknown	61, 62
Carbatrol (carbamazepine)	1997	Thought to block sodium channels, exact method unknown	40, 63
Depakote (divalproex sodium)	1996	Thought to inhibit GABA transaminase, thus increasing concentration of GABA. Also inhibits histone deacetylase 1 (HDAC1)	64, 65
Tegretol XR (carbamazepine)	1996	Thought to block sodium channels, exact method unknown	40, 63