Chapter 20
On the Development of New Drugs for the Treatment of Drug-Resistant Epilepsy: An Update on Different Approaches to Different Hypotheses

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Abstract Despite the continuous expansion of the available pharmacological options for the treatment of epilepsies and remarkable advances in understanding their pathophysiology, the proportion of refractory patients has remained roughly unchanged over the past 100 years.

In the last decade, hypotheses that try to explain the drug-resistant phenotype have increased in number and their scope has been more precisely specified, and some major advances related to some of these hypotheses have been realized, both at the preclinical and clinical levels. These include the use of gene therapies to revert the pharmacoresistant phenotype in animal models of epilepsy, advance into clinical trials and approval of tailored multitarget therapeutics (e.g., padsevonil and cenobamate) exhibiting encouraging results on refractory patients, approval of new drugs with new (and sometimes complex) mechanisms to address particularly severe and difficult-to-treat epileptic syndromes, and the first reports of applications of network analysis to rationally select combinations of antiseizure medications. The introduction of the *Epilepsy Therapy Screening Program* also constitutes a significant milepost that will possibly have a major impact on the development of new, more efficacious therapeutic options against epilepsy, as the focus of the international guidelines to screen for novel medications against epilepsy is now on refractory epilepsy and disease-modifying interventions.

This chapter, which intends to be a critical update of the one published back in the first edition of this volume, overviews the current hypotheses that intend to explain refractory epilepsy as well as plausible therapeutic strategies to address some of them.

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20.1 Drug-Resistant Epilepsy: Possible Explanations

According to the current definition of the International League Against Epilepsy (ILAE), the term *drug-resistant epilepsy* (often used interchangeably with intractable, pharmacoresistant, or refractory epilepsy) refers to "the failure of adequate trials of two tolerated and appropriately chosen and used antiepileptic drug schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom" (Kwan et al. 2010). Several dimensions must be examined when considering this definition. First, "adequate trials" implies that the therapeutic intervention has been applied at adequate strengths for a sufficient length of time. "Appropriately chosen" denotes that the chosen intervention has previously been demonstrated to be effective, preferably through randomized controlled trials, for the patient's epilepsy and seizure type. These aspects of the definition are not trivial at all. For instance, a pharmacological intervention that has been inappropriately selected according to the type of epilepsy will not be counted as one of the two (appropriate) interventions required by the definition before concluding that the patient is, in fact, drug resistant. Similarly, when a drug is withdrawn because of an adverse event before it has been titrated to its clinically effective dose range, thus not constituting an "adequate trial," it will not be counted as one of the specified interventions. These considerations are not only of utmost importance when deciding how a patient's disorder will be managed but also from a drug discovery perspective: some therapeutic interventions that could possibly achieve the target seizure-free status might be disregarded due to poor tolerability. Therefore, although the focus of this chapter is on therapeutic interventions addressing the underlying cause of pharmacoresistance, the development of new drugs for drug-resistant epilepsy should not exclude the need for safer, better-tolerated medications. It should also be noted that, at present, the terms antiseizure medications (ASMs) or antiseizure drugs (ASDs) are preferred over antiepileptic drugs to describe those pharmacological interventions that, in essence, are intended for symptomatic control (which does not exclude the possibility of beneficial effects on the course of the disease and comorbidities that result from downstream effects of seizures) but that have not demonstrated direct favorable actions on the underlying disease or its progression (Perucca et al. draft).

An increasing number of hypotheses have been raised to explain the origin of drug-resistant epilepsy (Tang et al. 2017; Bazhanova et al. 2021): the highly interrelated *transporter* and *pharmacokinetic hypotheses* (Löscher and Potschka 2005; Tang et al. 2017); the *target hypothesis* (Löscher and Potschka 2005; Schmidt and

Löscher 2005; Kwan and Brodie 2005; Remy and Beck 2006); *the gene variant hypothesis* (which may converge with the transporter, pharmacokinetic, and target hypotheses, as discussed later) (Cárdenas-Rodríguez et al. 2020); the *epigenetic hypothesis* (Kobow et al. 2013); the *intrinsic severity hypothesis* (Rogawski and Johnson 2008); the *neural network hypothesis* (Fang et al. 2011); and the *neuroinflammation hypothesis* (Löscher and Friedman 2020; Campos-Bedolla et al. 2022).

The *transporter hypothesis* suggests that drug resistance may arise from acquired activation or overexpression of efflux drug transporters that restrict drug distribution to the brain and/or parenchyma cells; such over-expression could occur at any of the cells of the neurovascular unit. The *pharmacokinetic hypothesis*, in essence complementary to the previous one, considers the role of efflux transporters outside the brain, and also the possible contribution of other drug clearance mechanisms, that is, biotransformation enzymes, to the insufficient bioavailability of ASMs. Research supporting the transporter hypothesis has focused on efflux transporters from the ATP-binding cassette (ABC) superfamily. Cumulative studies have revealed high expression levels of members of this superfamily, such as P-glycoprotein (Pgp), the breast cancer resistance protein (BCRP), and multidrug resistance protein (MRP), at the neurovascular unit of nonresponsive patients with epilepsy, either at the blood–brain barrier (BBB), glial cells and/or neurons (see, for instance, Tishler et al. 1995; Dombrowski et al. 2001; Sisodiya et al. 2002, 2006; Aronica et al. 2003, 2005; Lazarowski et al. 2004; Calatozzollo et al. 2005; Kubota et al. 2006; Ak et al. 2007). The lack of efficacy of those ASDs which are substrates of any of the upregulated efflux transporters could therefore be a consequence of the limited brain bioavailability of ASD (Marchi et al. 2005). However, in other cases, refractoriness has not been related to subtherapeutic concentrations specifically at the site of action, but to persistently low plasma levels of the drug due to enhanced plasma clearance, despite the administration of standard doses of ASMs (Lazarowski et al. 2004, 2007; Iwamoto et al. 2006; Czornyj et al. 2018). This could be related to the high expression levels of efflux transporters in other organs (e.g., intestines, liver, and kidney), which would restrict absorption and facilitate elimination (Tang et al. 2017). A valid question would be whether a therapeutic intervention can be regarded as an "adequate trial," thus contributing to the diagnosis of intractable epilepsy, if the chosen dose could not achieve therapeutic plasma concentrations. As exposed by Tang and collaborators (op. cit.), while it could be argued that abnormalities in ASD plasma concentrations would be readily captured by therapeutic drug monitoring, reference therapeutic plasma concentrations are not expected to be universally applied, and from a precision medicine perspective, it would be possibly better to define the target plasma concentration and to adjust ASD dosages accordingly on an individual basis. Interestingly, a series of recent articles by Lazaroswki et al. have provided grounds for the suggestive theory that overexpression of Pgp outside the brain may be causally related to heart failure and sudden unexpected death in epilepsy (Auzmendi et al. 2018, 2021; Akyuz et al. 2021; Czornyj et al. 2022), which, if confirmed, would add a new size to the pharmacokinetic hypothesis.

A general pharmacokinetic mechanism underlying drug-resistant epilepsy is consistent with the fact that the available ASMs act through a wide range of pharmacological targets. The transporter hypothesis has been fully validated in preclinical models of epilepsy. High levels of Pgp, associated with low brain bioavailability of its substrates, have been observed in animals with drug-resistant epilepsy, and the resistant phenotype has been reversed by co-administration of Pgp inhibitors (van Vliet et al. 2006; Brandt et al. 2006; Zhang et al. 2012). Conclusive evidence of the validity of the transporter hypothesis in humans, however, remains elusive, and the author's perspective is that interest in this hypothesis has diminished to some extent in recent years (possibly following disappointing clinical trials with second- and third-generation Pgp inhibitors in the field of oncology (Chung et al. 2016)), although some of the more recently proposed hypotheses provide mechanistic insight on how the increased expression of drug transporters is induced and regulated.

There are anecdotal cases (Summers et al. 2004; Ianetti et al. 2005; Schmitt et al. 2010; Pirker and Baumgartner 2011) and small-scale open-label studies (Asadi-Pooya et al. 2013; Narayanan et al. 2016) that showed improvement in patients with drug-resistant epilepsy when ASMs were co-administered with verapamil, a known Pgp inhibitor, but it is unclear whether the observed results are due to the intrinsic antiseizure activity of verapamil, Pgp inhibition, other effects on the drug pharmacokinetics, or more than one of these reasons. Using positron emission tomography (PET) and the PET ligand and Pgp-substrate (R)-[11C] verapamil with and without tariquidar (a selective Pgp inhibitor) in pharmacoresistant patients, Feldmann et al. (2013) corroborated the association between regionally localized Pgp overactivity and drug resistance patients with temporal lobe epilepsy. However, a small-scale randomized controlled trial showed no statistically significant decrease in seizure frequency in the pharmacoresistant patients receiving verapamil as adjuvant therapy; only 12 of the recruited patients completed the study (Borlot et al. 2014). Randomized controlled trials with selective inhibitors are needed to obtain definitive proof of the therapeutic potential of this theory.

The main argument against the transporter hypothesis is that, while several ASMs are proven substrates for ABC transporters, others are not (Zhang et al. 2012; Leandro et al. 2019); in fact, the evidence shows that the standard broad-spectrum ASD, valproic acid, is not transported by ABC carriers (Baltes et al. 2007; Leandro et al. 2019). As the transporter hypothesis has not been convincingly validated in clinical trials, current guidelines for the management of epilepsy do not consider the interaction with ABC transporters as a criterion for medication choice (Kanner et al. 2018; Park et al. 2019; Guery and Rheims 2021).

The *target hypothesis* proposes that compositional/structural (transcriptional or posttranscriptional) acquired alterations in the pharmacological targets of ASDs might explain the drug-resistant phenotype (Fattorusso et al. 2021; Fonseca-Barriendos et al. 2022). This hypothesis is based on reported loss of sensitivity to voltage-gated sodium channel blockers such as carbamazepine and phenytoin in patients and animal models of epilepsy (Schmidt and Löscher 2009). It has been observed that the inactivation effect of phenytoin on sodium channels is transiently

reduced in kindling models (Vreugdenhil and Wadman 1999), whereas the use-dependent effects of carbamazepine and phenytoin are permanently lost or reduced in the pilocarpine model of epilepsy and in some patients with temporal lobe epilepsy (Remy et al. 2003a, b; Jandová et al. 2006). Numerous changes in the expression of sodium channels subunits have been described in animal models of seizure and epilepsy and in patients with epilepsy (Bartolomei et al. 1997; Gastaldi et al. 1998; Aronica et al. 2001; Whitaker et al. 2001; Ellerkmann et al. 2003), suggesting that epileptogenesis and/or seizures may alter the ASDs targets. Mutations in the accessory subunit β1 have been linked to a dramatic loss in the use-dependent effect of phenytoin (Lucas et al. 2005). Furthermore, associations have been reported between alterations in GABAA receptor subunits and resistance to phenobarbital in animal models of temporal lobe epilepsy (Volk et al. 2006; Bethmann et al. 2008). The Achilles heel of the target hypothesis is that clinical ASMs associated with different modes of action exist, and even those ASDs that share a common mechanism (e.g., GABAA receptor allosteric modulators) sometimes bind to different binding sites of the same pharmacological target. Thus, the target hypothesis by itself would only satisfactorily explain the phenomenon of multidrug resistance involving drugs that share their mechanism and would be even less valid to explain resistance to drug combinations. However, as discussed in other sections of this chapter, some novel ASMs based on a multitarget strategy have shown encouraging results in drug-resistant patients. The outcome could be explained through the target hypothesis, but other possible explanations could be offered, as discussed in another chapter.

The *gene variant hypothesis* states that variants of genes involved in the pharmacodynamics and pharmacokinetics of ASMs or associated with the epileptic phenotype could be the source of drug resistance. It is clearly related to the transporter, pharmacokinetics, and target hypotheses, only that it specifies an intrinsic origin of the resistant phenotype rather than an acquired source of variability due to the course of the disorder and/or treatment. For instance, recent studies, including meta-analyses, have suggested an association between polymorphic variants of alpha and beta subunits of voltage-operated sodium channels and differences in their responsiveness to ASMs (e.g., Nazish et al. 2018; Bao et al. 2018; Zhang et al. 2021a; Li et al. 2021). In contrast, the *epigenetic hypothesis* argues that seizures may mediate epigenetic modifications resulting in persistent genomic methylation, histone density, posttranslational modifications, and noncoding RNA-based changes (Kobow et al. 2013). Liu et al. (2016) analyzed DNA methylation across the entire genome in brain tissue from ten drug-resistant patients and demonstrated the presence of several differentially methylated genes on the X chromosome and a significantly smaller number on the Y chromosome. Lv et al. (2019) investigated 75 Chinese patients (25 with CBZ-resistant epilepsy, 25 with CBZ-responsive epilepsy, and 25 controls) and found an association between methylation levels in the EPHX1 promoter and the CBZ-resistant phenotype.

The *intrinsic severity hypothesis* suggests that the inherent severity of the disorder is a key determinant of treatment outcomes (Rogawski and Johnson 2008). Epidemiological studies indicate that the single most important predictor of the

response to pharmacological interventions in epilepsy is the number of episodes at the initial stage of the disorder (MacDonald et al. 2000; Williamson et al. 2006; Sillampää and Schmidt 2006, 2009; Mohanraj and Brodie 2006; Kim et al. 2006; Hitiris et al. 2007). Recently, it has been suggested that the intrinsic severity hypothesis should be expanded to consider not only seizure frequency but also pathological high-frequency oscillations as an indicator of severity (Santana-Gomez et al. 2022). Some shortcomings of the intrinsic severity hypothesis have been underlined in the past (Schmidt and Löscher 2009): the lack of studies on the biological basis of disease severity; the lack of genetic studies comparing patients with low seizure frequency versus patients with high seizure frequency at the onset of the disorder; and the fact that there are reports of nonresponsive patients with low frequency of episodes in the early phase of epilepsy (Spooner et al. 2006). Some of these limitations are now being actively remedied through current sequencing technologies: sequencing-based studies on patients with nonlesional epilepsies have recently identified novel risk genes associated with severe epilepsies and revealed an excess of rare deleterious variation in less severe forms of epilepsy (Epi25 Collaborative 2019, Calhoun and Carvill 2020).

The *neural network hypothesis* states that adaptive remodeling of neural circuits induced by seizures may contribute to the development of drug-resistant epilepsy (Fang et al. 2011). Bearing in mind that remodeling of neural circuits also occurs in responsive patients, differences between the degree of neural reorganization in responsive and nonresponsive patients should be studied to support this latest explanation of drug resistance. In a recent perspective article discussing the need for a complex systems approximation to achieve a better understanding of drug resistance in epilepsy, Servilha-Menezes and Garcia-Cairasco (2022) underlined the fact that the occurrence of comorbid disorders in patients with epilepsy is associated with a negative prognosis regarding the chances of achieving and sustaining a seizure-free status. Interestingly, some common comorbid disorders with epilepsy, such as depression and anxiety, are also associated with abnormal neural networks/circuits (Duval et al. 2015; Oberlin et al. 2022) and, as importantly, with poor response to pharmacotherapy (Oberlin et al. 2022).

Finally, the *neuroinflammation hypothesis* suggests that inflammatory factors released during seizures can induce blood–brain barrier dysfunction (leaky vessels) and compensatory overexpression of efflux transporters, resulting in a loss of response to ASMs. Importantly, changes in microvascular permeability following seizures seemingly result in the increased transport of high-molecular-weight proteins (e.g., albumin), but not necessarily the free exchange of small ions or molecules (Kang et al. 2013). Consequently, unbound, pharmacologically relevant concentrations of ASDs in the brain may diminish (Marchi et al. 2009; Potschka et al. 2011). In other words, sub-efficacious unbound drug levels could arise from both reduced free drug levels due to complexation with albumin and increased expression of efflux pumps.

It is clear from the short precedent overview that refractory epilepsy is a complex, multifactorial phenomenon and that different hypotheses may explain the drug resistance phenomenon in different subgroups of patients (e.g., the gene variant

hypothesis would only apply to patients expressing the gene variants linked to drug resistance), whereas in some patients more than one hypothesis might be integrated to explain the resistant phenotype, or might exhibit some degree of overlap and convergence, as previously discussed by other authors (Schmidt and Löscher 2009; Servilha-Menezes and Garcia-Cairasco 2022). For instance, the transporter and pharmacokinetic hypotheses speak of a seizure- and/or treatment-induced activation of similar clearance mechanisms at the neurovascular unit or in organs outside the brain (e.g., liver and kidneys), whereas the gene variant hypothesis relates the activation of efflux and enzymatic biotransformation systems to genetic polymorphism (e.g., at regulatory regions of a gene). The neuroinflammation hypothesis provides a mechanistic explanation for the acquired overexpression of transporters at the blood–brain barrier and/or epileptic foci, as well as a complementary mechanism to explain reduced, subtherapeutic free drug levels in the brain parenchyma (extravasation of plasma proteins and sequestration of unbound drug).

In the following sections, we discuss some potential or current therapeutic approximations to address some of the previously overviewed hypotheses.

20.2 Possible Therapeutic Answers to the Transporter and Pharmacokinetic Hypothesis

The traditional hypothetical answer to overcome efflux transporter-mediated drug resistance was to develop therapeutic systems capable of evading or ameliorating the active efflux, either by inhibiting or downregulating ABC transporters, by hiding the ASDs from these systems (in a "Trojan horse" manner), or by designing novel ASDs without any affinity for ABC transporters. Potschka (2012) provided an excellent review on this matter.

The general strategies can then be synthesized as follows: (a) modulation of ABC transporters (i.e., inhibition and/or downregulation of transporters), (b) design of novel drugs which are not efflux transporter substrates, and (c) bypassing drug transport (or the Trojan horse strategy).

Most research on these strategies has focused on Pgp, the best-known representative of the ABC superfamily. However, several proteomic studies have shown that, in humans, the levels of BCRP at the neurovascular unit are comparable (if not higher) to those of Pgp (see Table 20.1) (Uchida et al. 2011; Shawahna et al. 2011; Al-Majdoub et al. 2019). Differences between cortical and subcortical tissues have also been observed (Huttunen et al. 2022). Moreover, numerous reports agree that the expression levels of different ABC transporters are interrelated, with direct and inverse co-expression patterns, depending on the case (Bordow et al. 1994; Choi et al. 1999; Cisternino et al. 2004; Bark et al. 2008; Miller et al. 2008). Since there is some degree of overlap across the substrates of different transporters, the possibility of upregulation of a given transporter to compensate for the disturbance of

Table 20.1 Expression levels of different ABC transporters found in brain microvessels across different quantitative proteomic studies (healthy brain tissue)

	Absolute protein expression levels (pmol/mg total protein)		
Transporter	Uchida et al. (2011)	Shawahna et al. (2011)	Al-Majdoub et al. (2019)
Pgp–ABCB1	2.85 (0.58)	3.98 (0.88)	2.58 (0.93)
MRP1–ABCC1	<0.21	<0.21	<0.05
MRP5–ABCC5	<0.50	<0.50	<0.01
MRP6–ABCC6	<0.17	<0.17	0.48 (0.06)
BCRP–ABCG2	6.06 (1.69)	6.15 (1.41)	2.22 (0.61)

another should be considered, especially when pursuing long-term therapeutic interventions, as in the case of epilepsy.

Initially, the inhibition of ABC transporters was intended with adjuvant administration of small-molecule inhibitors, as originally conceived in the field of oncology to deal with chemoresistance. Although nonclinical and initial clinical studies in the field of cancer treatment were promising at first, trials of first-, second-, and even third-generation agents have been terminated mostly due to serious safety issues (Deeken and Löscher 2007; Fox and Bates 2007; Lhommé et al. 2008; Tiwari et al. 2011). At this point, it is important to emphasize that ABC transporters comprise a concerted, complex efflux system with a prominent role in the disposal of waste products and toxins, and they also participate in the traffic of physiological compounds. Thus, permanent impairment or disruption is likely to result in severe side effects (again, one should bear in mind the chronic nature of epilepsy, which requires long-term treatment).

Recent research has focused on elucidating intracellular signaling pathways that control ABC transporters (their expression, intracellular trafficking, activation, and inactivation), such as those dependent on inflammatory stress and the activation of nuclear receptors. It has been proposed that identifying the molecular switches of these transporters will allow selective and transient modulation of transporter activity and/or expression for therapeutic purposes in different clinical scenarios (Hartz and Bauer 2010; Miller 2015), which includes turning the efflux mechanisms off for short, controlled periods. For instance, subchronic treatment with the cyclooxygenase-2 inhibitor SC-58236 blocked the status epilepticus-associated increase in Pgp expression in the lithium-pilocarpine status epilepticus model and enhanced the brain penetration of phenytoin (van Vliet et al. 2010). More recently, using siRNA, Yu et al. blocked inhibitory κ B kinase subunit β (IKKβ) gene transcription, which functions as an upstream regulator of inflammation and nuclear factor-kappa B activation (Yu et al. 2014). siRNA targeting IKKβ was delivered to rats before seizure induction by kainic acid, abolishing Pgp overexpression and decreasing seizure susceptibility in epileptic rats. Enrique et al. reported a mouse model of drug-resistant seizures based on the subchronic administration of proconvulsant doses of 3-mercaptopropionic acid (Enrique et al. 2017). Reduced sensitivity to known Pgp substrate ASDs (phenytoin and phenobarbital) was observed; such a loss of response was not extended to non-substrates of Pgp, such as carbamazepine, diazepam, or

levetiracetam. Loss of sensitivity was reversed by co-administration of the Pgp inhibitor nimodipine, and Pgp overexpression was observed in the cerebral cortex, hippocampus, and striatum of the animals. This model was later used for screening new drugs capable of reversing the drug-resistant phenotype (Enrique et al. 2021). A virtual screening campaign was implemented with a focus on compounds that could simultaneously elicit anticonvulsant and anti-inflammatory effects. The underlying rationale was that treatment with such multitarget compounds would block Pgp upregulation induced by glutamate and pro-inflammatory signals. Subchronic administration of one of the in silico hits, sebacic acid, during the seizure-induction period was able to revert the overexpression of Pgp similarly to celecoxib. Although the anti-inflammatory effects of the virtual screening hits were not validated, this study seems to be conceptually in line with the transporter hypothesis as well as the neuroinflammation hypothesis. A similar study was conducted by Liu et al. (2022) who found that antioxidant preventive treatment with N-acetylcysteine also prevented the development of resistance.

An alternative strategy that could provide delivery of a drug to the brain without the toxic issues associated with the impairment of efflux mechanisms involves the identification of novel ASDs that are not recognized by ABC transporters (Demel et al. 2008, 2009). Such an approximation implies the use of ABC transporters as antitargets. Review articles on the use of structure- and ligand-based approaches to detect substrates for Pgp and other ABC pumps have been published in the past (Klepsch et al. 2014; Montanari and Ecker 2015); more recent studies on the subject have relied on modern machine learning approximations such as adaptive learning (Cerruela García and García-Pedrajas 2018), ensemble learning (Hou et al. 2020), and deep learning (Zhang et al. 2021b), among others. Couyoupetrou et al. (2017) described the implementation of a virtual screening campaign to identify anticonvulsant drugs with no substrate liability for Pgp. Four of the chosen hits were tested in a bidirectional transport assay using an MDCK II- MDR I cell monolayer. The efflux ratios obtained in the presence and absence of amiodarone (a Pgp inhibitor) showed no significant differences, confirming the lack of significant Pgp-mediated efflux at the assayed concentration. Similarly, Gantner et al. (2017) proposed BCRP as an antitarget in a virtual screen exercise and identified four anticonvulsant agents with no affinity for such transporter.

The last strategy oriented to bypassing the biochemical barrier posed by efflux transporters involves the use of a carrier system (e.g., a nanocarrier or a prodrug) to "hide" the drug from the efflux system. Additionally, it should be emphasized that the targeting of nanoparticulated systems might be favored in leaky vessels; accordingly, drug delivery to the brain through pharmaceutical nanocarriers could also be linked to the neuroinflammation hypothesis. Moreover, drug administration via routes or delivery methods that avoid or minimize the first-pass effect or that protect the drug from elimination mechanisms could also be used in relation to the pharmacokinetic and gene variant hypothesis.

A wide variety of nanosystems have been studied to enhance permeability to the brain, especially in the field of oncology, whereas the degree of advancement for other neurological disorders seems to lag slightly (Sim et al. 2020; Hersh et al.

2022). An exhaustive overview of these studies lies outside the scope of this chapter. Regarding the specific application of this strategy to encapsulate ASDs, different nanosystems have been studied for the delivery of clonazepam, diazepam, phenytoin, ethosuximide, 5–5-diphenyl hydantoin, carbamazepine, valproic, oxcarbazepine, phenobarbital, and NMDA receptor antagonists, among others (Fresta et al. 1996; Kim et al. 1997; Jeong et al. 1998; Nah et al. 1998; Darius et al. 2000; Friese et al. 2000; Thakur and Gupta 2006; Abdelbary and Fahmy 2009; Varshosaz et al. 2010; Eskandari et al. 2011; Scioli Montoto et al. 2018, 2021, 2022). A central question would be whether these pharmaceutical technology artifices are capable of improving the bioavailability of drugs in the central nervous system and, if so, the molecular basis of such improvement. Unfortunately, most of these reports limit their scope to the physical characterization and in vitro behavior of the reported systems. Nevertheless, some of them have explored the in vivo behavior with variable results. Darius et al. (2000) found that brain tissue levels of valproic acid were not significantly modified by administration inside nanoparticles, although the nanosystem was found to reduce drug metabolism via mitochondrial beta-oxidation. Friese et al. (2000) reported that poly(butyl cyanoacrylate) nanoparticles coated with polysorbate 80 extended the duration of the anticonvulsive activity of the NMDA receptor antagonist MRZ 2/576, presumably by preventing active transport processes. Eskandari et al. (2011) observed an enhanced protective effect of valproic acid in the maximal electroshock seizure (MES) test when the drug was administered within nanostructured lipid carriers. Intranasal administration of 4 mg/kg of the encapsulated drug led to almost three-fold higher brain concentrations than an intranasally administered solution of 30 mg/kg of valproic acid, and the brain–plasma ratio was also increased through the nanocarrier. Scioli Montoto et al. (2018) reported that protection from seizures by carbamazepine incorporated into a nanostructured lipid carrier remained for at least 2 h after intraperitoneal administration, but there was no difference from the free drug group.

Prodrugs are another option to circumvent the blood–brain barrier, sometimes making use of uptake transporters from the solute carrier (SLC) superfamily (e.g., dopamine is administered as its precursor l-dopa, which is transported into the brain by the l-type amino acid transporter and metabolized to release dopamine in situ) (Mandaya et al. 2010). Numerous prodrugs of different anticonvulsant agents such as phenytoin, gabapentin, valproic acid, and eslicarbazepine have been developed with the goal of improving bioavailability by modifying drug absorption, distribution, and/or elimination (Bennewitz and Saltzman 2009; Trojnar et al. 2004; Bialer and Soares-da-Silva 2012). For example, DP-VPA (Fig. 20.1) was conceived to be specifically activated at the epileptic foci. In it, a molecule of valproic acid is linked to lecithin, leading to a 50-fold increase in efficacy in the pentylenetetrazol-induced seizures test (Trojnar et al. 2004).

Noteworthy, in the last decades, it has been proven that many pharmaceutical excipients which are usually incorporated into pharmaceutical delivery systems can inhibit or modulate ABC transporters' function through different mechanisms (Bansal et al. 2009; Nguyen et al. 2021). For example, it has been proposed that PEG and surfactants, such as sorbitans and polysorbates, can disrupt the lipid

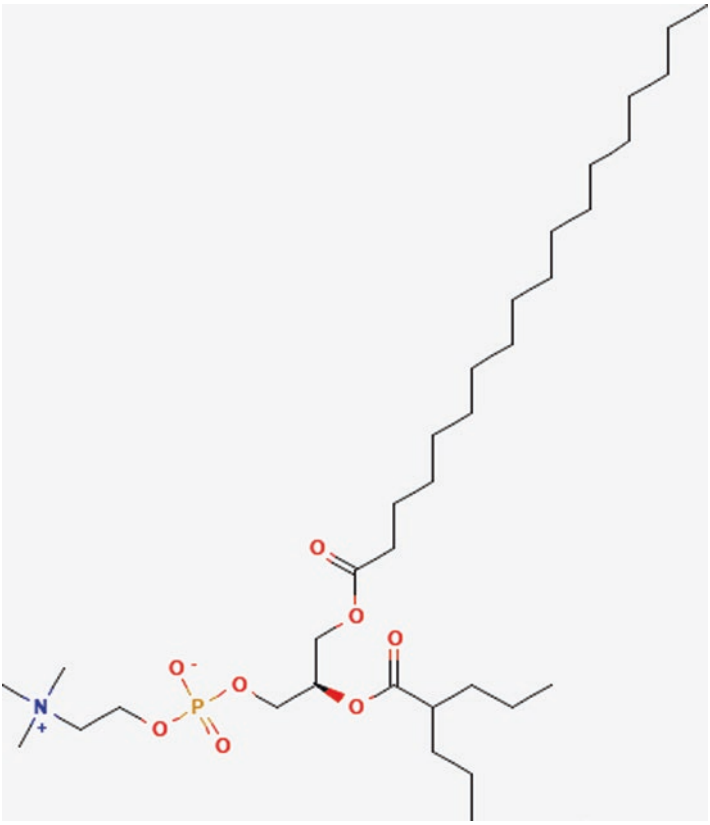

Fig. 20.1 DP-VPA, a prodrug of valproic acid, has been investigated as a potential treatment for severe forms of epilepsy, including status epilepticus, acute repetitive seizures in children, bipolar disorder, and migraine prophylaxis

arrangement of the cellular membrane and that these perturbations have been shown to modulate Pgp activity (Lo 2003). This kind of modulation is interesting because it may increase drug bioavailability in a transient manner, without the undesired effects of direct inhibition. Besides their possible role in modulating transporters, cumulative evidence indicates that nanoparticles' coating leads to the adsorption of elements from the blood, such as apolipoproteins, which in turn would allow distribution to the brain by receptor-mediated transcytosis (Wohlfart et al. 2012 and references therein).

20.3 Possible Therapeutic Answers to the Target Hypothesis

Several (if not most) central nervous system disorders present a complex etiology that includes a combination of polygenic, environmental, and neurodevelopmental factors. Empirical evidence with treatments for mood disorders from the

phenotypic-screening era (e.g., antidepressants) shows that searching for polyspecific, selective nonselective drugs (multitarget-directed ligands, multitarget drugs, polyvalent drugs, hybrid drugs, or "magic shotguns") may prove a safer and more efficacious way to address such complexity than the development of highly selective, single-target drugs (Roth et al. 2004; Margineanu 2016). There are abundant examples of recent developments in the field of central nervous system therapeutics based on this relatively new paradigm, including drugs in development for Alzheimer's and Parkinson's diseases (Cavalli et al. 2008; Youdim and Buccadfasco 2005), depression, schizophrenia, and others (Decker and Lehmann 2007; Wong et al. 2010).

There are many reasons why multitarget therapies are also of most interest within the field of epilepsy. Empiric evidence has suggested that—if total drug load is carefully monitored— some refractory patients may achieve seizure remission on polypharmacy, especially if the pharmacologic properties of the specific ASDs being combined are considered (Canevini et al. 2010; Kwan and Brodie 2006). Second, the recent introduction of ASDs with novel (fenfluramine) or complex (cannabidiol) modes of action has proven successful in particularly resistant, severe, and catastrophic epileptic syndromes, such as Dravet and Lennox-Gastaut (Devinsky et al. 2018; Balagura et al. 2020; Scheffer et al. 2021). Third, many currently used ASDs are unintended multitarget agents selected through phenotypic models of seizures (Bianchi et al. 2009). Fourth, the design of tailored multitarget ASDs sounds like a natural answer to the target hypothesis of drug resistance, considering that it is unlikely that two distinct drug targets will lose sensitivity to drugs simultaneously. The benefits of targeting more than one rationally selected target can also be achieved by drug combinations chosen from a network pharmacology perspective. Combination therapies for epilepsy are covered in a separate chapter of this volume, which deals with epilepsy and complexity.

Two of the most recently developed drugs for refractory epilepsy Refractory epilepsy (RE) are, in fact, tailored multitarget agents. Cenobamate (Fig. 20.2) is a dual agent that acts on voltage-operated sodium channels and as an allosteric positive modulator of the $GABA_A$ receptor. A post hoc analysis of a subset of patients from a long-term multicenter phase 3 open-label study showed high rates of sustained 100% and ≥90% seizure reduction. Almost half of the patients who decided to continue with cenobamate after the study was finalized achieved seizure freedom for at least 12 months (Fig. 20.3) (Sperling et al. 2021). Noteworthy, the patients enrolled in the study had been diagnosed with focal epilepsy and had previously failed to achieve seizure freedom despite being treated with stable doses of 1–3 ASMs.

Encouraging results were also obtained in a phase 2a, randomized, placebo-controlled, double-blind (3 weeks) plus open-label (8 weeks) multicenter study of padsevonil (Fig. 20.4) as an add-on therapy (padsevonil being another dual-acting agent which acts through SV2s and as a partial, low-affinity allosteric modulator of GABAA receptor) (Muglia et al. 2020). The study enrolled refractory patients with focal epilepsy who had failed to control seizures with four or more ASDs regimens of adequate dose and duration. During the blind period, patients in the treatment

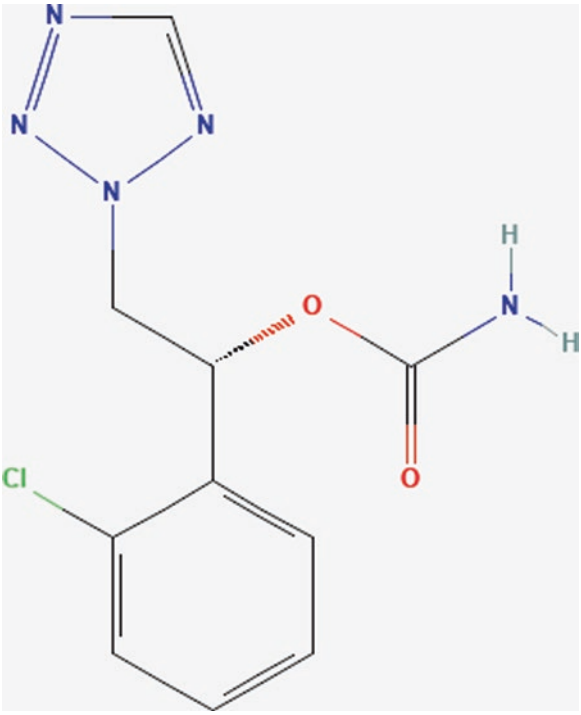

Fig. 20.2 Cenobamate: a recently approved dual-acting ASM with encouraging results as add-on therapy in patients with drug-resistant epilepsy

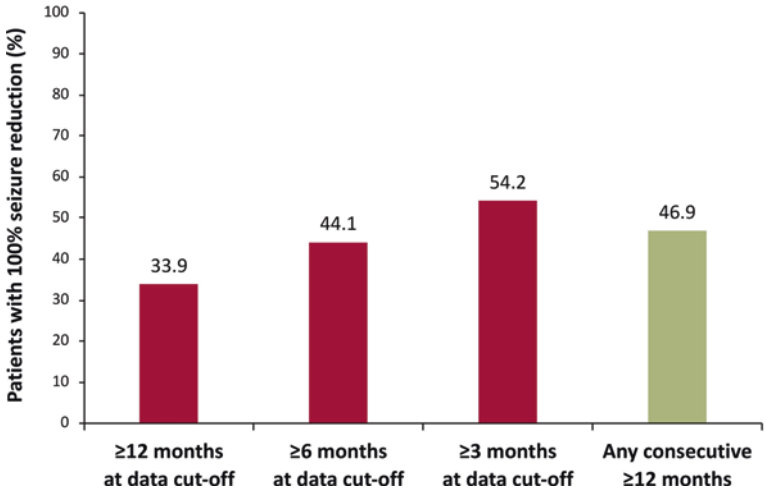

Fig. 20.3 Among the patients that remained on cenobamate as add-on therapy after a phase 3 large-scale open-label study, 46.9% achieved seizure freedom for at least 12 months. (Reproduced from Sperling et al. (2021) under a Creative Commons license)

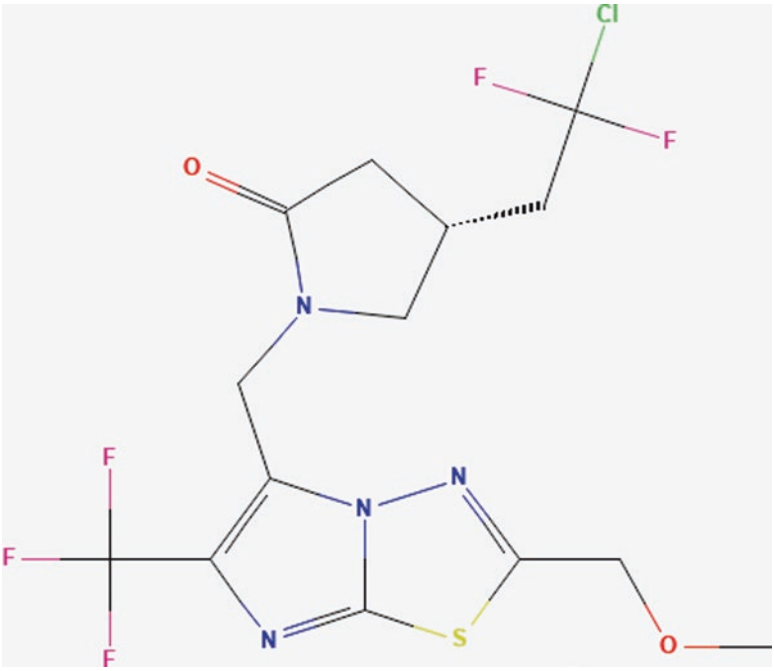

Fig. 20.4 Padsevonil is a dual-acting ASD in development, which has shown promising results in drug-resistant epileptic patients participating in a phase 2a study

group rapidly achieved seizure reduction of approximately 50%, whereas no clear benefit was observed in the placebo group. When switched to treatment, seizure reduction was also observed in the placebo group. Remarkably, 76% of the patients chose to remain on padsevonil treatment after the study ended, reflecting the positive perception on the benefits of the intervention. Later, however, a phase 2b study failed to demonstrate the superiority of padsevonil (Contreras-García et al. 2022).

20.4 Conclusions

In recent years, the number of hypotheses that aim to explain the drug-resistant phenomenon in epilepsy has expanded, and new ideas have expanded the horizon of the classical tentative explanations to the resistant phenotype. It is possible that no single hypothesis may explain all cases of refractory epilepsy, and the available explanations partially overlap and/or converge in many cases.

Among the strategies proposed to cope with drug-resistant epilepsies associated with genetic or acquired upregulation of brain and/or peripheral transporters, drug design of new ASDs with no substrate liability for ABC transporters appears as a reasonably safe option. Circumventing transport by either prodrug design or

encapsulation or conjugation of ASDs with nanodelivery systems seem also as a good alternative. Noteworthy, the neuroinflammation hypothesis of drug-resistant epilepsy suggests that the delivery of pharmaceutical nanocarriers to the brain could be enhanced by passive targeting of the seizure-induced leaky vessels. Considering the physiologic (and critical) role of efflux transporters, downregulating their activity to basal levels should be preferred, due to safety reasons, to fully abolish their function. Interestingly, seizure models that achieve overexpression of efflux transporters at the brain capillaries have been reported and might be of help to screen for novel therapeutics that can prevent or reverse the resistant phenotype.

On the other hand, innovative ASDs with complex pharmacology, in line with a systems biology perspective, have been successfully introduced to the market in the last few years or are under investigation for the treatment, as add-on therapies, of drug-resistant epilepsies, with encouraging results at clinical trials.

The increasing knowledge of how oxidative stress and inflammation contribute to a negative circle (where seizures induce changes that contribute to the occurrence of new seizures) opens new paths to the development of new treatments that might be of special value when facing epilepsies characterized with severe and frequent seizures.

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