Chapter 14
On the Development of New Antiepileptic Drugs for the Treatment of Pharmacoresistant Epilepsy: Different Approaches to Different Hypothesis

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Abstract Despite the introduction of 15 new antiepileptic drugs to the market since 1990, around a third of the epileptic patients do not achieve seizure remission with current known medications. The chapter overviews current hypothesis on the causes of drug resistant epilepsy, with an emphasis on the most documented explanations. On the basis of those hypotheses, current approaches to the development of novel antiepileptic medications are overviewed, including adjuvant Pgp-inhibitors, development of Pgp-non substrates, use of nanocarriers to circumvent active transport, design of multi-target directed ligands and adjuvant therapies with antioxidant and anti-inflammatory medications. In line with current discussions on the matter, it is proposed that different hypothesis may serve as explanation for different subgroups of drug-resistant patients, and that—in the light of recent basic research—at least some of the hypotheses may be interrelated.

Keywords Refractory epilepsy • Drug resistant epilepsy • Antiepileptic drugs • Drug design • Transporter hypothesis • Target hypothesis • Intrinsic severity hypothesis • ABC transporters • Multi-target directed drugs • Nanocarriers

14.1 Refractory Epilepsy: Current Explanations

According to the current definition from the International League Against Epilepsy (ILAE) the term refractory (or intractable, or drug resistant) epilepsy refers to the failure of adequate trials of two tolerated, appropriately chosen antiepileptic drug schedules (either as monotherapies or in combination) to achieve sustained seizure

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freedom (Kwan et al. 2010). In the previous definition "appropriate" indicates an intervention that has previously been shown to be effective (preferably in randomized controlled studies) for the patient's epilepsy and seizure type, while "adequate" indicates that the drug has been administered at adequate dosage for a sufficient length of time. Regarding what constitutes and adequate period without seizures for a patient to be regarded as "seizure-free," a minimum of three times the longest pre-intervention inter-seizure period or 12 months (whichever is longer) has been proposed. Application of a standardized definition of refractoriness is not trivial, since depending on the definition chosen the frequency of drug resistant epilepsy varies considerably (between 10 and nearly 40 %) (Beleza 2009). What is more, as discussed later, general applicability of a given hypothesis of drug resistant epilepsy may critically depend on what we actually call "drug resistant epilepsy."

Despite the fact that there are currently more than 20 available antiepileptic drugs (AEDs) and that 15 third generation agents have been introduced to the market since 1990, the clinical need of refractory epilepsy remains unmet (Bialer 2012; Löscher and Schmidt 2011): there are still no solid evidence indicating improved efficacy. There are currently four hypotheses explaining the nature of refractory epilepsy: on the one hand, the traditional transporter and target hypothesis (Löscher and Potschka 2005; Schmidt and Löscher 2005; Kwan and Brodie 2005; Remy and Beck 2006); more recently, the inherent severity hypothesis and the neural network hypothesis have also been proposed (Rogawski and Johnson 2008; Fang et al. 2011). Among them, the transporter hypothesis is so far, without a shadow of doubt, the most extensively studied.

The transporter hypothesis suggests that intractable epilepsy may have a pharmacokinetic basis. It states that drug resistance may emerge, as in other disorders, from intrinsic or acquired activation or over-expression of drug transporters involved in drug distribution, metabolism and elimination. Research supporting this hypothesis has focused in efflux transporters from the ATP-binding cassette (ABC) superfamily. Evidence abounds indicating high expression levels of members of this family such as P-glycoprotein (Pgp), breast cancer resistance protein (BCRP), and multidrug resistance proteins (MRPs) at the neurovascular unit of nonresponsive patients (either at the blood–brain barrier or glial cells or neurons) (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). Lack of efficacy of those AEDs which are substrates of any of the up-regulated efflux transporter would be a consequence of limited bioavailability of the therapeutic agent in the brain or specifically at the epileptic focus. In fact, some studies showed reduced AEDs concentrations in the brain extracellular fluid and epileptic tissue of refractory patients (Marchi et al. 2005; Rambeck et al. 2006). A general pharmacokinetic mechanism underlying refractory epilepsy is consistent with the fact that available AEDs act through a wide range of molecular mechanisms. The transporter hypothesis has been fully verified in animal models of epilepsy. Several animal models of epilepsy (chronic models) have provided evidence of Pgp over-expression in brain tissue from animals with refractory epilepsy (Zhang et al. 2012), and drug resistance has been reverted by co-administration of Pgp inhibitors together

with the AED (van Vliet et al. 2006; Brandt et al. 2006). Nevertheless, conclusive evidence of the validity of the transporter hypothesis in humans remains elusive. There are some (anecdotical) cases of patients who have showed improvement when AED were co-administered with Verapamil, a known Pgp-inhibitor (Summers et al. 2004; Ianetti et al. 2005; Schmitt et al. 2010; Pirker and Baumgartner 2011). It is still not clear, however, if the observed results are due to the intrinsic antiepileptic activity of verapamil, to Pgp inhibition or another effect on AEDs pharmacokinetics, and randomized control trials with more selective inhibitors are needed to obtain definitive proof of concept. The main argument against the transporter hypothesis is the fact that numerous but not all AEDs are substrates of human Pgp (Zhang et al. 2012). At this point one should bear in mind that current definition of drug resistant epilepsy requires only two adequate, appropriate, well-tolerated AED interventions to consider that a patient presents refractory epilepsy. It is then conceivable that (if the transporter hypothesis were valid) a patient would be diagnosed as drug resistant if at least one of those two AEDs interventions does not include a Pgp-non-substrate (e.g., Carbamazepine). It has been suggested that the transporter hypothesis may be valid for a subgroup of the epileptic patients (Löscher and Delanty 2009).

The target hypothesis states that structural (transcriptional or posttranscriptional) alterations in AEDs molecular targets might explain pharmacoresistance. This hypothesis is based, essentially, in 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), while the use-dependent effect of Carbamazepine and Phenytoin is permanently lost or reduced in the pilocarpine model and in temporal lobe epilepsy patients (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 epileptic patients (Bartolomei et al. 1997; Gastaldi et al. 1998; Aronica et al. 2001; Whitaker et al. 2001; Ellerkmann et al. 2003), suggesting seizures or epileptogenesis may alter AEDs targets. Mutations at accessory subunit β1 have been linked to a dramatic loss in the use-dependent effect of phenytoin (Lucas et al. 2005). On the other hand, associations between alterations at $GABA_A$ receptor subunits and resistance to phenobarbital in animal models of temporal lobe epilepsy have been reported (Volk et al. 2006; Bethmann et al. 2008). The main objection to the target hypothesis is that, as has been already mentioned, there exist clinical AEDs associated to different mechanisms of action. Even those AEDs that share a common mechanism (e.g., $GABA_A$ receptor allosteric modulators) frequently bind to different sites of the same receptor. Thus, the target hypothesis by itself would only satisfactorily explain the phenomenon of multidrug resistance involving drugs that share their mechanism of action.

A third hypothesis, the hypothesis of the intrinsic severity, proposes the inherent severity of the disorder as determinant of the treatment outcome (Rogawski and Johnson 2008). It relies on epidemiologic data which indicates that the single most

important factor linked to the prognosis of epilepsy is the number of episodes at the early phase of the disorder (MacDonald et al. 2000; Williamson et al. 2006; Sillampää and Schmidt 2006; Mohanraj and Brodie 2006; Kim et al. 2006; Hitiris et al. 2007; Sillampää and Schmidt 2009). Some limitations of the intrinsic severity hypothesis have been highlighted (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 disorder onset and; the fact that there are reports of nonresponsive patients with low frequency of episodes at the early phase of epilepsy (Spooner et al. 2006).

Very recently, a fourth hypothesis has arisen. The neural network hypothesis states that the adaptive remodeling of neural circuits that follows seizures may contribute to the development of refractory epilepsy. However, one should remember that remodeling of neural circuits also occurs in responsive patients. Therefore, differences between the degree of neural reorganization in responsive and nonresponsive patients should be studied to support this latest explanation to drug resistance.

This short overview suggests that either different hypothesis may explain the drug resistance phenomenon in different subgroups of patients (understanding that refractory epilepsy is a complex, multi-factor phenomenon and conceiving that in some patients more than one factor may be present simultaneously) or that the previous hypothesis may be integrated (Schmidt and Löscher 2009), with the two first hypothesis (partially) providing a biological basis for the others. Most importantly to the scope of this chapter, different hypothesis claim for different strategies to develop novel therapeutic answers. In the next sections we discuss potential implications of the first three hypothesis in the field of AEDs development.

14.2 Possible Therapeutic Answers to the Transporter Hypothesis

The obvious answer to overcome efflux transporter-mediated drug resistance is to develop therapeutic systems to circumvent this barrier to achieving adequate concentrations of the drug in its site of action. An excellent review on this matter has recently been published (Potschka 2012). The general strategies studied in the last 15 years to overcome ABC transporters can be synthesized as (Talevi and Bruno-Blanch 2012): (a) modulation of ABC transporters (i.e., reversal of multidrug resistance and down-regulation of transporters); (b) design of novel drugs which are not efflux transporter-substrates; (c) bypassing drug transport (or the Trojan horse strategy). Most of the research on these strategies has focused on the best known representative of the ABC superfamily, Pgp (note that Pgp was purified back in 1979 and it was not until 1990s that MRPs were identified). However, it is now established that there exist numerous transporters involved in transport of endogenous and exogenous compounds and that the levels of expression of different ABC transporters are interrelated (in some cases, a co-expression pattern has been observed; in others, an inverse relationship has been established) (Miller et al. 2008; Cisternino

et al. 2004; Bark et al. 2008; Choi et al. 1999; Bordow et al. 1994). Taking into consideration that the spectra of substrates of different ABC transporters overlap to a certain degree, it might be hypothesized that up-regulation of a given transporter might have a compensatory role in the transient or permanent disturbance of other, which might explain the observed development of tolerance to some interventions aimed at regulating Pgp function (van Vliet et al. 2006). One must consider that development of tolerance is not acceptable when dealing with long-term drug treatments such as AEDs.

Regarding transporters modulation, the most advanced research relates to add-on therapies of specific inhibitors of ABC transporters, a strategy that was originally conceived for cancer treatment. Although preclinical and initial clinical results in the field of cancer treatment were encouraging at first, trials of first, second and even third generation agents had to be stopped at clinical stage due to serious adverse effects (Deeken and Löscher 2007; Lhommé et al. 2008; Tiwari et al. 2011; Fox and Bates 2007). These results have called into question the general validity of this approach of overcoming cellular drug resistance by the use of transporters inhibitors, even though trials continue in order to find more effective and safe inhibitors for Pgp and other transporters (Deeken and Löscher 2007; Akhtar et al. 2011). At this point it is important to remember that ABC transporters comprise a concerted, complex efflux and influx dynamic system whose substrates are not only drugs but also endogenous compounds (e.g., waste products) and toxins. They are implicated in the inflammatory response to several stress and harmful stimuli, and, apparently, they have a role in neurodegenerative diseases such as Alzheimer's and Parkinson's disease (Hartz and Bauer 2010). Thus, their permanent impairment or disruption is likely to result in severe side effects (again, one should bear in mind the chronic nature of epilepsy, which demands long-term treatment). A similar outcome to RNA interference technologies to down-regulate a given gene codifying a member of the ABC superfmailiy may be expected (Potschka 2012). Recent research has then focused on elucidating intracellular signaling pathways that control ABC transporters (their expression, intracellular trafficking, activation and inactivation). It is proposed that finding the molecular switches of these transporters will allow selective modulation of transporters function and or expression for therapeutic purposes in different clinical scenarios (Hartz and Bauer 2010), which includes turning the efflux mechanisms off for short, controlled periods of time.

Another strategy which should provide delivery of a drug to the brain without the toxic issues associated to the impairment of the efflux transport is virtual screening or computer-aided design of novel AEDs which are not recognized by ABC transporters (Demel et al. 2008, 2009). A review on in silico models for early detection of Pgp substrates has been recently published (Chen et al. 2012). A 2D QSAR model to detect anti-maximal electroshock seizures (MES) drug candidates (Talevi et al. 2007a, b, 2012), an ensemble of 2D models to identify Pgp-susbtrates (Di Ianni et al. 2011) and a structure-based approach based on homology modeling of human Pgp were jointly applied in a virtual screening campaign to ZINC and DrugBank databases (Irwin and Shoichet 2005; Knox et al. 2011). From 360 compounds predicted as Pgp-non-substrates anticonvulsants, ten diverse candidates

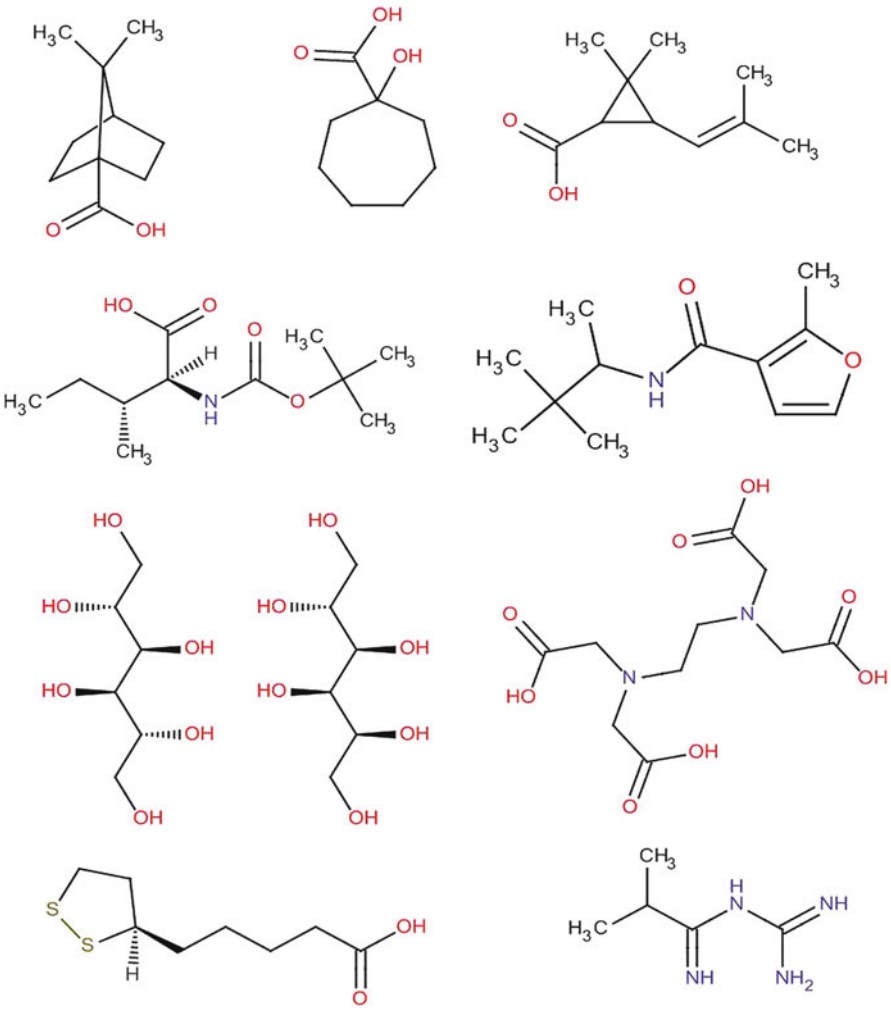

Fig. 14.1 A series of novel anticonvulsants emerging from a multistep virtual screening campaign aiming at novel treatments for refractory epilepsy

(Fig. 14.1) were acquired and tested in the MES test, with good results (Di Ianni et al. 2012, submitted).

The last strategy implies the application of a carrier system to "hide" the drug from the efflux pump. Different carrier systems have been tested to increase the bioavailability of drugs to the brain, among them nanosystems (polymer nanoparticles, nanogels, lipid nanocapsules, liposomes) (Bansal et al. 2009; Patel et al. 2009; Bennewitz and Saltzman 2009; Alam et al. 2010,). An exhaustive review of all the carriers that have been tested in brain drug targeting to avoid recognition by transporters will deserve an entire chapter or even a book, so we are including some

Table 14.1 Increment of brain bioavailability of VPA when administered intranasally with nanostructured lipid carriers [adapted from Eskandari et al. (2011)]

Formulation	Route	Dose (mg/kg)	Plasma concentration 60 min after administration (μg/ml), mean ± SD	Brain concentration 60 min after administration (μg/g) mean ± SD	Brain:plasma ratio
NLC of VPA	Intranasal	4	7.96 ± 2.9	64.35 ± 5.7	8.4
NLC of VPA	IP	20	11.35 ± 5.8	19.85 ± 8.5	1.65
Sodium VPA solution	Intranasal	30	3.87 ± 1.9	23.36 ± 8.3	6.77
Sodium VPA solution	IP	150	275.85 ± 39.5	112 ± 16	0.42

NLC nanostructured lipid carriers, *VPA* valproic acid

examples for illustrative purposes. A 60-fold increase in the brain localization of doxorubicin (a known Pgp-substrate) in rats, when administered i.v. as polysorbate 80-coated nanoparticles (compared to: i.v. administration in saline solution; in polysorbate 80 solution and; bound to nanoparticles without polysorbate 80 coating) (Gulyaey et al. 1999). Much more recently, a 2.6-fold increase in coumarin-6 localization in the brain through encapsulation of the drug in poly(ε-caprolactone)-block-poly(ethyl ethylene phosphate) nanomicelles was achieved (Zhang et al. 2010). Regarding specific application of this strategy to antiepileptic agents, different nanosystems have been studied for the delivery of Clonazepam, Diazepam, Phenytoin, Ethosuximide, 5-5-diphenyl hydantoin, carbamazepine, and valproic acid (VPA) and NMDA receptor antagonists (Fresta et al. 1996; Kim et al. 1997; Jeong et al. 1998; Nah et al. 1998; Ryu et al. 2000; Darius et al. 2000; Friese et al. 2000; Thakur and Gupta 2006; Abdelbary and Fahmy 2009; Varshosaz et al. 2010; Eskandari et al. 2011). A valid question would be whether this galenic artifices do improve availability of the drug in the central nervous system (CNS) and, if so, the molecular basis of such improvement. Unfortunately, most of this reports limit to physical characterization and in vitro behavior of the proposed systems. However, some of them explore the in vivo behavior of the nanosystems, with variable results. Darius et al. (2000) found that the brain tissue levels of VPA were not altered by administration with nanoparticles, though the nanosystem inhibits metabolism of VPA via mitochondrial beta-oxidation. Friese et al. (2000) reported that poly(butylcyanoacrylate) nanoparticles coated with polysorbate 80 prolong the duration of the anticonvulsive activity of NMDA receptor antagonist MRZ 2/576, presumably by prevention of active transport processes at the choroid plexus. More recently, Eskandari et al. (2011) have found an increased protective effect of VPA in the MES test when the drug was administered in nanostructured lipid carriers to rats. Intranasal administration of a dose of 4 mg/kg of nanostructured lipid carriers of VPA lead to almost three times higher brain concentrations than an intranasally administered solution of 30 mg/kg of the drug; brain–plasma ratio was also increased with the nanosystem (Table 14.1).

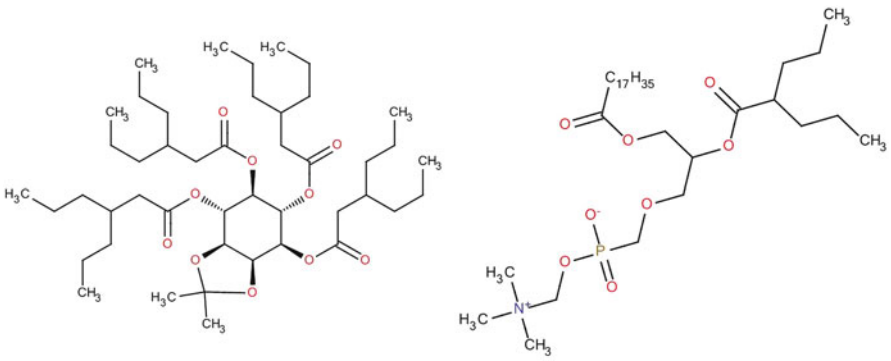

Fig. 14.2 Two prodrugs of VPA designed for improvement of VPA bioavailability at the epileptic focus: a prodrug of myo-inositol (*left*) and DP-VPA (*right*)

Prodrugs are another option to circumvent the blood–brain barrier, sometimes making use of influx transporters (e.g., dopamine is administered as its precursor L-dopa, which is transported into the brain by the L-type amino acid transporter and bio-transformed to dopamine in situ) (Mandaya et al. 2010). Numerous prodrugs of different anticonvulsant agents such as phenytoin, gabapentin, VPA and eslicarbazepine have been developed in order to improve bioavailability by regulation of drug absorption, distribution and elimination (Bennewitz and Saltzman 2009; Trojnar et al. 2004; Bialer and Soares-da-Silva 2012). DP-VPA (Fig. 13.2) was designed to be specifically activated at the epileptic focus. It is a prodrug of VPA in which the VPA moiety is covalently bound to a phospholipid, lecithin, leading to a 50-fold increase in efficacy in the pentylenetetrazol-induced seizures test (Trojnar et al. 2004). Similarly, our group has developed prodrugs of VPA with myo-inositol (Fig. 14.2) aiming at capitalizing the active influx of inositol enantiomers into the brain; the activity of these prodrugs in animal models of seizure is also increased compared to VPA, seemingly by improving CNS bioavailability (Bodor et al. 2000; Moon et al. 2007; Bruno-Blanch and Moon 2010). Whether these prodrugs interact with efflux transporters and bypass up-regulated transporter molecules at the neurovascular unit has yet to be studied.

It is noteworthy that in the last few years it has been proven that, besides helping bypassing Pgp, many pharmaceutical excipients which are usually incorporated into carrier-systems can inhibit or modulate Pgp function by different mechanisms (Bansal et al. 2009). For example, it has been proposed that PEG and surfactans such as sorbitans and polysorbates can disrupt the lipid arrangement of the cellular membrane and that these perturbations have been shown to modulate Pgp activity (Lo 2003). This kind of modulation is interesting since it may increase drug bioavailability in a transient manner, without the undesired effects of direct inhibition. Besides its possible role modulating transporters, cumulative evidence indicates that nanoparticle's coating leads to adsorption of elements from the blood such as apolipoproteins, which in turn allows distribution to the brain by receptor-mediated transcytosis (Wohlfart et al. 2012 and references therein).

14.3 Possible Therapeutic Answers to the Target Hypothesis

Several CNS disorders (either neurological or affective) present a complex etiology which includes a combination of polygenic, environmental, and neuro-developmental factors. Empiric evidence with effective treatments for some of such diseases (e.g., antidepressants) shows that searching for polyspecific, selective non-selective drugs (multi-target directed-ligands or "magic shotguns" or polyvalent drugs) may prove more safe and effective than the development of highly selective, single-target drugs (Roth et al. 2004). There are plenty examples of recent developments in the field of CNS medications based on this new paradigm, including developing drugs for Alzheimer and Parkinson's diseases (Cavalli et al. 2008; Youdim and Buccadfasco 2005), schizophrenia, depression and other mood disorders (Decker and Lehmann 2007; Wong et al. 2010).

There are many reasons why multi-target therapies are attractive in the field of epilepsy. First, evidence indicate that—if total drug load is carefully watched—some refractory patients may achieve seizure remission on poly-pharmacy, especially if the pharmacologic properties of the specific AEDs being combined is taken into account (Canevini et al. 2010; Kwan and Brodie 2006). A recent study on 131 patients who underwent successful epilepsy surgery seems to indicate that, at least in the early postoperative stage, dual-therapy may be more effective than monotherapy to achieve seizure remission (Zeng et al. 2012). Second, the normal function of neural networks may be more likely preserved by multiple small adjustments than by a single, strong perturbation, reducing not only the likelihood of central side-effects but also the induction of counter-regulatory processes which may relate with drug resistance (Löscher and Schmidt 2011; Bianchi et al. 2009). What is more: many currently used AEDs are in fact unintended multi-target agents (Bianchi et al. 2009).

In the light of the evidence that refractoriness may be in some cases related to modifications in drug targets, the design of novel multi-target AEDs seems as a natural answer to the second hypothesis of drug resistance, considering that it seems to be less likely that two distinct drug targets are altered simultaneously. Therefore, even if one target of a multi-target drug has lost sensitivity, one can speculate that the other/s will remain sensitive.

From the drug design perspective, in silico, rational approaches to develop multifunctional agents can be classified in two strategies (Ma et al. 2010). On the one hand, the combinatorial approach, in which parallel Virtual Screening searches against each target of interest are conducted, retaining those hits that simultaneously gather all the structural requisites needed to interact with each individual target. In other words, the common hits from parallel Virtual Screening searches (one for every model associated to a particular target) are retained. In the background of multi-target drug discovery, the Virtual Screening for ligands for each individual target must be highly sensitive (i.e., a reduced number of false negatives should be observed) since the collective retrieval rate for multiple targets will tend to be relatively low than when aiming to individual targets (one might speculate that,

naturally, it is more difficult to find compounds that selectively interact with different targets without being excessively promiscuous). In contrast, when drugs that selectively interact with a single target are being searched, in certain contexts one might sacrifice sensitivity in order to gain specificity. The second strategy is the fragment-based approach. Here, multiple elements or scaffolds that bind to each of the targeted targets are combined (usually through a linker) into a single, often larger molecule. The main drawback of this later approach relates to the poorer pharmacokinetic and toxicological profile of the final drug. Unless small, highly specific blocks/fragments are combined, it is unlikely that a given compound will gather the features for a CNS drug-like drug.

14.4 Possible Therapeutic Answers to the Intrinsic Severity Hypothesis

If the intrinsic severity hypothesis was valid, AEDs research would face to elemental questions. Firstly, what are the determinants of epilepsy severity? And, if the answer to that initial question was answered, how could one control, through a therapeutic intervention, such determinants? During the last 10 years, basic research has begun to provide us some knowledge to attempt some very draft answers to these issues.

Acquired epilepsy is typically initiated by a brain insult followed by a latent, silent period whereby molecular, biochemical and cellular alterations occur in the brain and eventually lead to chronic epilepsy (Waldbaum and Patel 2010a). In the last 10–15 years a link between epileptogenesis and oxidative stress, mitochondrial impairment and inflammation has been established by a large body of studies (Waldbaum and Patel 2010b; Waldbaum et al. 2010; Devi et al 2008; Liang and Patel 2006; Shin et al. 2008; Patel 2004; Sudha et al 2001; Vezzani and Granata 2005; Vezzani et al. 2011; Choi and Koh 2008). These phenomena seem to be both cause and consequence of seizures, constituting a vicious circle which results in a chronic disorder, e.g., inflammatory mediators are released during seizures, and inflammatory mediators take part in seizure generation and exacerbation. It is also interesting to note that chronic inflammation and oxidative unbalance take part in the physiopathology of a diversity of neurological disorders. The brain combines a peculiar set of factors which makes it particularly vulnerable to reactive species: high rate of oxidative metabolism, low antioxidant defenses and abundant polyunsaturated lipids (Devi et al 2008).

In line with the integrative approach towards explaining refractory epilepsy, a series of studies developed in the last decade agree that pro-inflammatory signals and Reactive Oxygen Species play a role in the regulation of ABC transporters' expression and activity. For example, exposing isolated rat brain capillaries to nanomolar concentrations of ET-1 and TNF-α for long periods of time (above 4 h) increased Pgp-mediated transport compared to control levels, and after a 6-h

exposure Pgp transport was roughly doubled (Bauer et al. 2007). Von Wedel-Parlow et al. (2009) reported that Pgp levels were increased by TNF-α within 6 h but decreased later (Von Wedel-Parlow et al. 2009). Poller et al. reported similar results working with a human cell line of immortalized brain microvessels endothelial cells; they also noted that IL-6 treatment produced a slight decrease in Pgp mRNA expression (Poller et al. 2010). Regarding the influence of Reactive Oxygen Species on efflux transporters expression levels, the first evidence of up-regulation of Pgp came from in vitro experiments on primary culture of rat brain endothelial cells (Felix and Barrand 2002). Four hours after exposure to 100 μM H_2O_2, up-regulation of Pgp was observed at both mRNA and protein levels, which continue to increase up to a maximum at 48 h. A biphasic up-regulation was also observed after a 6-h hypoxia and subsequent reoxigenation (H/R) treatment; in this case, return to basal levels was observed following reoxigenation by 48 h. More recently, Robertson et al. (2009) reproduced the previous experiments comparing the effects of H_2O_2 H/R treatments in primary rat brain endothelial cells and immortalized rat brain endothelial cells. Although the production of Reactive Oxygen Species after H_2O_2 was more pronounced in immortalized cells lines, similar up-regulation of Pgp, at the protein level, was observed after the oxidative stress treatments in both types of cells. Similar results were obtained with other models, such as exposure to diesel exhaust particles or glutathione depletion (Hartz et al. 2008; Hong et al. 2006; Wu et al. 2009).

The discovery of the role of pro-inflammatory mediators and oxidative stress in epilepsy explains current interest in immune, antiinflammatory and neuroprotective therapies as potential strategies to improve disease prognosis. For example, it was observed that ascorbic and lipoic acids ameliorate oxidative stress in experimental seizures (Santos et al. 2009; Militão et al. 2010). ACTH—a peptide that releases endogenous steroids in the patient—is used as a treatment for infantile spasms, a childhood refractory epilepsy; its efficacy has been confirmed in controlled trials (Pellock et al. 2010), while the use of other anti-inflammatory therapies such as steroids remains controversial due to current lack of controlled clinical studies (Vezzani et al. 2011).

14.5 Conclusions

There are currently four different hypotheses for drug resistant epilepsy. None of them seems to completely explain all cases of refractory epilepsy, but subgroups of unresponsive patients instead. At first sight, each of them claims for a different therapeutic approach. Among the strategies proposed to overcome transporter-mediated refractory epilepsy, computer-aided research on new AEDs which are not recognized by ABC transporters, and circumventing transport by either prodrug design or nanoscale drug carriers seem as the best alternatives. Considering the efflux transporters' role in the disposal of potentially toxic endogenous and exogenous compounds, we do not believe adjuvant inhibitory therapies as a feasible

option in the case of long-term treatments (e.g., AEDs). Still, one should consider that inhibition of a given transporter is often compensated by up-regulation of another member of the ABC superfamiliy. Regarding the target hypothesis, design of multi-target agents that introduce mild perturbations to several AED targets seems to be a good alternative for the treatment of those patients with certain altered, unsensitive target. Finally, considering the intrinsic severity hypothesis, and since inflammation and oxidative stress seem to have a role in generation and exacerbation of seizures, controlled trials on the possible effects of antioxidants, immune and anti-inflammatory medication on epilepsy may have an impact on disease prognosis and severity, and consequently improve the chance of seizure remission.

Recent findings on the effect of oxidative stress and inflammation on ABC transporters expression confirm the idea that some (if not all) of the hypothesis of drug resistant epilepsy can be integrated. More research on the relationship between oxidative stress and alterations to AED targets should be explored. Revealing the fine mechanisms that govern biochemical pathways and cellular events involved in epileptogenesis (e.g., angiogenesis, inflammation) would create new opportunities for the development of innovative antiepileptic medications.

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References

Abdelbary G, Fahmy RH. Diazepam-loaded solid lipid nanoparticles: design and characterization. AAPS Pharm Sci Tech. 2009;10:211–9.

Ak H, Ay B, Tanrivedi T, Sanus GZ, Is M, Sar M, et al. Expression and cellular distribution of multidrug resistance-related proteins in patients with focal cortical dysplasia. Seizure. 2007;16:493–503.

Akhtar N, Ahad A, Khar RK, Jaggi M, Ágil M, Igbal Z, Ahmad FJ, Talegaonkar S. The emerging role of P-glycoprotein inhibitors in drug delivery: a patent review. Expert Opin Ther Pat. 2011;21:561–76. doi: 10.1517/13543776.2011.561784.

Alam MI, Beg S, Samad A, Baboota S, Kohli K, Ali J, et al. Strategy for effective brain drug delivery. Eur J Pharm Sci. 2010;40:385–403.

Aronica E, Yankaza B, Troost D, van Vliet FA, Lopes da Silva FH, Gorter JA. Induction of neonatal sodium channel II and III alpha-isoform mRNAs in neurons and microglia after status epilepticus in the rat hippocampus. Eur J Neurosci. 2001;13:1261–6.

Aronica E, Gorter JA, Jansen GH, van Veelen CW, van Rijen PC, Leenstra S, et al. Expression and cellular distribution of multidrug transporter proteins in two major causes of medically intractable epilepsy: focal cortical dysplasia and glioneuronal tumors. Neuroscience. 2003;118:417–29.

Aronica E, Gorter JA, Redeker S, van Vliet EA, Ramkema M, Scheffer GL, et al. Localization of breast cancer resistance protein (BCRP) in microvessel endothelium of human control and epileptic brain. Epilepsia. 2005;46:849–57.

Bansal T, Akhtar N, Jaggi M, Khar RK, Talegaonkar S. Novel formulation approaches for optimizing delivery of anticancer drugs based on P-glycoprotein modulation. Drug Discov Today. 2009;14:1067–74.

Bark H, Xu HD, Kim SH, Yun J, Choi CH. P-glycoprotein down-regulates expression of breast cancer resistance protein in a drug-free state. FEBS Lett. 2008;582:2595–600.
Bartolomei F, Gastaldi M, Massacrier A, Planells R, Nicolas S, Cau P. Changes in the mRNAs encoding subtypes I, II and III sodium cannel alpha subunits following kainate-induced seizures in rat brain. J Neurocytol. 1997;26:667–78.
Bauer B, Hartz AM, Miller DS. Tumor necrosis factor alpha and endothelin-1 increase P-glycoprotein expression and transport activity at the blood–brain barrier. Mol Pharmacol. 2007;71:667–75.
Beleza P. Refractory epilepsy: a clinically oriented review. Eur Neurol. 2009;62:65–71.
Bennewitz MF, Saltzman, WM. Nanotechnology for delivery of drugs to the brain for epilepsy. Neurotherapeutics. 2009;6:323–36. doi: 10.1016/j.nurt.2009.01.018.
Bethmann K, Fritschy JM, Brandt C, Löscher W. Antiepileptic drug resistant rats differ from drug responsive rats in $GABA_A$ receptor subunit expression in a model of temporal lobe epilepsy. Neurobiol Dis. 2008;31:169–87.
Bialer M. Chemical properties of antiepileptic drugs (AEDs). Adv Drug Deliv Rev. 2012;64:887–95.
Bialer M, Soares-da-Silva P. Pharmacokinetics and drug interactions of eslicarbazepine acetate. Epilepsia. 2012;53:935–46.
Bianchi MT, Pathmanathan J, Cash SS. From ion channels to complex networks: magic bullet versus magic shotgun approaches to anticonvulsant pharmacotherapy. Med Hypotheses. 2009;72:297–305.
Bodor N, Moon SC, Bruno-Blanch LE. Synthesis and pharmacological evaluation of prodrugs of valproic acid. Pharmazie. 2000;55:184–6.
Bordow SB, Haber M, Madafigli J, Cheung B, Marshall GM, Norris MD. Expression of the multidrug resistance-associated protein (MRP) gene correlates with amplification and overexpression of the N-myc oncogene in childhood neuroblastoma. Cancer Res. 1994;54:5036–40.
Brandt C, Nethmann K, Gastens AM, Löscher W. The multidrug transporter hypothesis of drug resistance in epilepsy: proof-of-principle in a rat model of temporal lobe epilepsy. Neurobiol Dis. 2006;24:202–11.
Bruno-Blanch L, Moon SC. US Patent 7,763,650; 2010.
Calatozzollo C, Gelati M, Ciusani E, Sciacca FL, Pollo B, Cajola L, et al. Expression of drug resistance proteins Pgp, MRP1, MRP3, MRP5 and GST.pi in human glioma. J Neurooncol. 2005;74:113–21.
Canevini MP, De Sarro G, Galimberti CA, Gatti G, Licchetta L, Malerba A, et al. Relationship between adverse effects of antiepileptic drugs, number of coprescribed drugs, and drug load in a large cohort of consecutive patients with drug-refractory epilepsy. Epilepsia. 2010;51:797–804.
Cavalli A, Bolognesi ML, Minarini A, Rosini M, Tumiatti V, Recanatini M, et al. Multi-target-directed ligands to combat neurodegenerative diseases. J Med Chem. 2008;51:347–72.
Chen L, Li Y, Yu H, Zhang L, Hou T. Computational models for predicting substrates or inhibitors of P-glycoprotein. Drug Discov Today. 2012;17:343–51.
Choi J, Koh S. Role of brain infalmmation in epileptogenesis. Yonsei Med J. 2008;49:1–18.
Choi CH, Kim SH, Rha HS, Jeong JH, Park YH, Min YD, et al. Drug concentration-dependent expression of multidrug resistance-associated protein and P-glycoprotein in the doxorubicin-resistant acute myelogenous leukemia sublines. Mol Cell. 1999;9:314–9.
Cisternino S, Mercier C, Bourasset F, Roux F, Scherrmann JM. Expression, upregulation, and transport activity of the multidrug-resistance protein abcg2 at the mouse blood–brain barrier. Cancer Res. 2004;64:3296–301.
Darius J, Meyer FP, Sabel BA, Schroeder U. Influence of nanoparticles on the brain-to-serum distribution and the metabolism of valproic acid in mice. J Pharm Pharmacol. 2000;52:1043–7.
Decker M, Lehmann J. Agonistic and antagonistic bivalent ligands for serotonin and dopamine receptors including their transporters. Curr Top Med Chem. 2007;7:347–53.

Deeken JF, Löscher W. The blood–brain barrier and cancer: transporters, treatment and Trojan horses. Clin Cancer Res. 2007;13:1663–74.

Demel MA, Schwha R, Krämer O, Ettmayer P, Haaksma EE, Ecker GF. In silico prediction of substrate properties for ABC-mulridrug transporters. Expert Opin Drug Metab Toxicol. 2008;4:1167–80.

Demel MA, Krämer O, Ettmayer P, Haaksma EE, Ecker GF. Predicting ligand interactions with ABC transporters in ADME. Chem Biodivers. 2009;6:1960–9.

Devi PU, Manocha A, Vohora D. Seizures, antiepileptics, antioxidants and oxidative stress: an insight for researchers. Expert Opin Pharmacother. 2008;9:3169–77.

Di Ianni M, Talevi A, Castro EA, Bruno-Blanch LE. Development of a highly specific ensemble of topological models for early identification of P-glycoprotein substrates. J Chemometr. 2011;25:313–22.

Di Ianni M, Enrique A, Palestro P, Gavernet L, Talevi A, Bruno-Blanch L. Several new diverse anticonvulsant agents discovered in a virtual screening campaign aime at novel antiepileptic drugs to treat refractory epilepsy. J Chem Inf Model. 2012;52:3325–30. doi: 10.1021/ci300423q.

Dombrowski SM, Desai SY, Marroni M, Cucullo L, Goodrich K, Bingaman W, et al. Overexpression of multiple drug resistance genes in endothelial cells from patients with refractory epilepsy. Epilepsia. 2001;42:1501–6.

Ellerkmann RK, Remy S, Chen J, Sochiyko D, Elger CE, Urban BW, et al. Molecular and functional changes in voltage-dependent Na(+) channels following pilocarpine-induced status epilepticus in rat dentrate granule cells. Neuroscience. 2003;119:323–33.

Eskandari S, Varshosaz J, Minaiyan M, Tabbakhian M. Brain delivery of valproic acid via intranasal administration of nanostructured lipid carriers: in vivo pharmacodynamic studies using rat electroshock model. Int J Nanomedicine. 2011;6:363–71.

Fang M, Xi ZQ, Wu Y, Wang XF. A new hypothesis of drug refractory epilepsy: neural network hypothesis. Med Hypotheses. 2011;76:871–6.

Felix RA, Barrand MA. P-glycoprotein expression in rat brain endothelial cells: evidence for regulation by transient oxidative stress. J Neurochem. 2002;80:64–72.

Fox E, Bates SE. Tariquidar (XR9576): a P-glycoprotein drug efflux pump inhibitor. Expert Rev Anticancer Ther. 2007;7:447–59.

Fresta M, Cavallaro G, Giammona G, Wehrli E, Puglisi G. Preparation and characterization of polyethyl-2-cyanoacrylate nanocapsules containing antiepileptic drugs. Biomaterials. 1996;17:751–8.

Friese A, Seiller E, Quack G, Lorenz B, Kreuter J. Increase of the duration of the anticonvulsive activity of a novel NMDA receptor antagonist using poly(butylcyanoacrylate) nanoparticles as a parenteral controlled release system. Eur J Pharm Biopharm. 2000;49:103–9.

Gastaldi M, Robaglia-Schlupp A, Massacrier A, Planells R, Cau P. mRNA coding for voltage-gated sodium channel beta2 subunit in rat central nervous system: cellular distribution and changes following kainate induced seizures. Neurosci Lett. 1998;249:53–6.

Gulyaey AE, Gelperina SE, Skidan IN, Antropoy AS, Kivman GY, Kreuter J. Significant transport of doxorubicin into the brain with polysorbate 80-coated nanoparticles. Pharm Res. 1999;16:1564–9.

Hartz AM, Bauer B. Regulation of ABC transporters at the blood–brain barrier: new targets for CNS therapy. Mol Interv. 2010;10:293–304.

Hartz AM, Bauer B, Block ML, Hong JS, Miller DS. Diesel exhaust particles induce oxidative stress proinflammatory signaling and P-glycoprotein up-regulation at the blood–brain barrier. FASEB J. 2008;22:2723–33.

Hitiris N, Mohanraj R, Norrie J, Sills GJ, Brodie MJ. Predictors of pharmacoresistant epilepsy. Epilepsy Res. 2007;75:192–6.

Hong H, Lu Y, Ji ZN. Up-regulation of P-glycoprotein expression by glutathione depletion-induced oxidative stress in rat brain microvessel endotelial cells. J Neurochem. 2006;98:1465–73.

Ianetti P, Spalice A, Parisi P. Calcium-channel blocker verapamil administration in prolonged and refractory status epilepticus. Epilepsia. 2005;46:967–9.
Irwin JJ, Shoichet BK. ZINC – a free database of commercially available compounds for virtual screening. J Chem Inf Model. 2005;45:177–82.
Jandová K, Päsler D, Leite Antonio L, Raue C, Ji S, Njunting M, et al. Carbamazepine-resistance in the epileptic dentate gyrus of human hippocampal slices. Brain. 2006;129:3290–306.
Jeong YI, Cheon JB, Kim SH, Nah JW, Lee YM, Sung YK, et al. Clonazepam release from core-shell type nanoparticles *in vitro*. J Control Release. 1998;51:169–78.
Kim HJ, Jeong YI, Kim SH, Lee YM, Cho CS. Clonazepam release from core-shell type nanoparticles *in vitro*. Arch Pharm Res. 1997;20:324–9.
Kim LG, Johnson TL, Marson AG, Chadwick DW, Medical Research Council MESS Study Group. Predicting risk of seizure recurrence after a single seizure and early epilepsy: further results from the MESS trial. Lancet Neurol. 2006;5:317–22.
Knox C, Law V, Jewison T, Liu P, Ly S, Frlokis A, et al. DrugBank: a comprehensive resource for "omics" research on drugs. Nucleic Acid Res. 2011;39(Database Issue):D1035–41.
Kubota H, Ishihara H, Langmann T, Schmitz G, Stieger B, Wieser HG, et al. Distribution and functional activity of P-glycoprotein and multidrug resistance-associated proteins in human brain microvascular endothelial cells in hippocampal sclerosis. Epilepsy Res. 2006;68: 213–28.
Kwan P, Brodie MJ. Potential role of drug transporters in the pathogenesis of medically intractable epilepsy. Epilepsia. 2005;46:224–35.
Kwan P, Brodie MJ. Combination therapy in epilepsy: when and what to use. Drugs. 2006;66:1817–29.
Kwan P, Arzimanoglou A, Berg AT, Brodie MJ, Allen Hauser W, Mathern G, et al. Definition of drug resistant epilepsy: consensus proposal by the *ad hoc* task force of the ILAE Comission on therapeutic strategies. Epilepsia. 2010;51:1069–77.
Lazarowski A, Massaro M, Schteinschnaider A, Intruvini S, Sevlever G, Rabinowicz A. Neuronal MDR-1 gene expression and persistent low levels of anticonvulsants in a child with refractory epilepsy. Ther Drug Monit. 2004;26:44–6.
Lhommé C, Joly F, Walker JL, Lissoni AA, Nicoletto MO, Manikhas GM, et al. Phase III study of valspodar (PSC 833) combined with paclitaxel and carboplatin compared with paclitaxel and carboplatin alone in patients with stage IV or suboptimally debulked stage III epithelial ovarian cancer or primary peritoneal cancer. J Clin Oncol. 2008;26:2674–82.
Liang LP, Patel M. Seizure-induced changes in mitochondrial redox status. Free Radic Biol Med. 2006;40:16–22.
Lo YL. Relationships between the hydrophilic–lipophilic balance values of pharmaceutical excipients and their multidrug resistance modulating effect in Caco-2 cells and rat intestines. J Control Release. 2003;90:37–48.
Löscher W, Delanty N. MDR1/ABCB1 polymorphisms and multidrug resistance in epilepsy: in and out of fashion. Pharmacogenomics. 2009;10:711–3.
Löscher W, Potschka H. Role of drug efflux transporters in the brain disposition and treatment of brain diseases. Prog Neurobiol. 2005;76:22–76.
Löscher W, Schmidt D. Modern antiepileptic drug development has failed to deliver: ways out of the current dilemma. Epilepsia. 2011;52:667–78.
Lucas PT, Meadows LS, Nicholls J, Ragsdale DS. An epilepsy mutation in the beta1 subunit of the voltage-gated sodium channel results in reduced channel sensitivity to Phenytoin. Epilepsy Res. 2005;64:77–84.
Ma X, Shi Z, Tan C, Jiang Y, Go ML, Low BC. *In silico* approaches to multi-target drug discovery. Computer aided multi-target drug design, multi-target virtual screening. Pharm Res. 2010;27:739–49.
MacDonald NK, Johnson AL, Goodridge DM, Cockerell OC, Sander JW, Shorvon SD. Factors predicting prognosis of epilepsy after presentation with seizures. Ann Neurol. 2000;48:833–41.

Mandaya N, Oberoi RK, Minocha M, Mitra AK. Transporter targeted drug delivery. J Drug Deliv Sci Technol. 2010;20:89–99.

Marchi N, Guiso G, Rizzi M, Pirker S, Novak K, Czech T, Baumgastner C, Janigro D, Caccia S, Vezzani A. Pilot study on brain-to-plasma partition of 10,11-Dyhydro-10-hydroxy-5H-dibenzo(b,f)azepine-5-carboxamide and MDR1 brain expression in epilepsy patients not responding to oxcarbazepine. Epilepsia. 2005;46:1613–9. doi: 10.1111/j.1528-1167.2005.00265.x.

Militão GC, Ferreira PM, de Freitas RM. Effects of lipoic acid on oxidative stress in rat striatum after pilocarpine-induced seizures. Neurochem Int. 2010;56:16–20.

Miller DS, Bauer B, Hartz AMS. Modulation of P-glycoprotein at the blood–brain barrier: opportunities to improve CNS pharmacotherapy. Pharmacol Rev. 2008;60:196–209.

Mohanraj R, Brodie MJ. Diagnosing refractory epilepsy: response to sequential treatment schedules. Eur J Neurol. 2006;13:277–82.

Moon SC, Echeverria GA, Punte G, Ellena J, Bruno-Blanch LE. Crystal structure and anticonvulsant activity of (+/−)-1,2:4,5-di-O-isopropylidene-3,6-di-O-(2-propylpentanoyl)-myoinositol. Carbohydr Res. 2007;342:1456–61.

Nah JW, Paek YW, Jeong YI, Kim DW, Cho CS, Kim SH, et al. Clonazepam release from poly(DL-lactide-co-glycolide) nanoparticles prepared by dialysis method. Arch Pharm Res. 1998;21:418–22.

Patel M. Mitochondrial dysfunction and oxidative stress: cause and consequence of epileptic seizures. Free Radic Biol Med. 2004;37:1951–62.

Patel MM, Goval BR, Bhadada SV, Bhatt JS, Amin AF. Getting into the brain: approaches to enhance brain drug delivery. CNS Drugs. 2009;23:35–58.

Pellock JM, Hrachovy R, Shinnar S, Baram TZ, Bettis D, Dlugos DJ, et al. Infantile spasms: A U.S. consensus report. Epilepsia. 2010;51:2175–89.

Pirker S, Baumgartner C. Termination of refractory focal status epilepticus by the P-glycoprotein inhibitor verapamil. Eur J Neurol. 2011;18:e151.

Poller B, Drewe J, Krähenbühl S, Huwyer J, Gutmann H. Regulation of BCRP (ABCG2) and P-glycoprotein (ABCB1) by cytokines in a model of the human blood–brain barrier. Cell Mol Neurobiol. 2010;30:63–70.

Potschka H. Role of CNS efflux drug transporters in the antiepileptic drug delivery: overcoming CNS efflux drug transport. Adv Drug Deliv Rev. 2012;64:896–910.

Rambeck B, Jürgens UH, May TW, Pannek HW, Behne F, Ebner A, Gorij A, Straub H, Speckmann EJ, Pohlmann-Eden B, Löscher W. Comparison of brain extracellular fluid, brain tissue, cerebrospinal fluid, and serum concentrations of antiepileptic drugs measured intraoperatively in patients with intractable epilepsy. Epilepsia. 2006;47:681–94. doi: 10.1111/j.1528-1167.2006.00504.x.

Remy S, Beck H. Molecular and cellular mechanisms of pharmacoresistance in epilepsy. Brain. 2006;129:18–35.

Remy S, Gabriel S, Urban BW, Dietrich D, Lehmann TN, Elger CE, et al. A novel mechanism underlying drug resistance in chronic epilepsy. Ann Neurol. 2003a;53:469–79.

Remy S, Urban BW, Elger CE, Beck H. Anticonvulsant pharmacology of voltage-gated Na+ channels in hippocampal neurons of control and chronically epileptic rats. Eur J Neurosci. 2003b;17:2648–58.

Robertson SJ, Kania KD, Hladky SB, Barrand MA. P-glycoprotein expression in immortalized rat brain endothelial cells: comparisons following exogenously applied hydrogen peroxide and after hypoxia-reoxygenation. J Neurochem. 2009;111:132–41.

Rogawski MA, Johnson MR. Intrinsic severity as a determinant of antiepileptic drug refractoriness. Epilepsy Curr. 2008;8:127–30.

Roth BL, Sheffler DJ, Kroeze WK. Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. Nat Rev Drug Discov. 2004;3:353–9.

Ryu J, Jeong YI, Kim IS, Lee JH, Nah JW, Kim SH. Clonazepam release from core-shell type nanoparticles of poly(epsilon-caprolactone)/poly(ethyleneglycol)/poly(epsilon-caprolactone) triblock copolymers. Int J Pharm. 2000;200:231–42.

Santos IM, Tomé Ada R, Saldanha GB, Ferreira PM, Militão GC, Freitas RM. Oxidative stress in the hippocampus during experimental seizures can be ameliorated with the antioxidant ascorbic acid. Oxid Med Cell Longev. 2009;2:214–21.

Schmidt D, Löscher W. Drug resistance in epilepsy: putative neurobiologic and clinical mechanisms. Epilepsia. 2005;46:858–77. doi:10.1111/j.1528-1167.2005.54904.x.

Schmidt D, Löscher W. New developments in antiepileptic drug resistance: and integrative view. Epilepsy Curr. 2009;9:47–52.

Schmitt FC, Dehnicke C, Merschhemke M, Meencke HJ. Verapamil attenuates the malignant treatment course in recurrent status epilepticus. Epilepsy Behav. 2010;17:565–8.

Shin EJ, Ko KH, Kim WK, Chae JS, Yen TP, Kim HJ, et al. Role of glutathione peroxidase in the ontogeny of hippocampal oxidative stress and kainate seizure sensitivity in the genetically epilepsy-prone rats. Neurochem Int. 2008;52:1134–47.

Sillampää M, Schmidt D. Natural history of treated childhood onset epilepsy: prospective long-term population based study. Brain. 2006;129:617–24.

Sillampää M, Schmidt D. Early seizure frequency and aetiology predict long-term medical outcome in childhood-onset epilepsy. Brain. 2009;132:989–98.

Sisodiya SM, Lin WR, Harding BN, Squier MV, Thorn M. Drug resistance in epilepsy: expression of drug resistance proteins in common causes of refractory epilepsy. Brain. 2002;125:22–31.

Sisodiya SM, Martinian L, Scheffer GL, van der Valk P, Scheper RJ, Harding BN, et al. Vascular colocalization of P-glycoprotein, multidrug resistance associated protein 1, breast cancer resistance protein and major vault protein in human epileptogenic pathologies. Neuropathol Appl Neurobiol. 2006;32:51–63.

Spooner CG, Berkovik SF, Mitchell LA, Wrennall JA, Harvey AS. New-onset temporal lobe epilepsy in children: lesion on MRI predicts poor seizure outcome. Neurology. 2006;67:2117–8.

Sudha K, Rap AV, Rao A. Oxidative stress and antioxidants in epilepsy. Clin Chim Acta. 2001;303:19–24.

Summers MA, Moore JL, McAuley JW. Use of verapamil as a potential P-glycoprotein inhibitor in a patient with refractory epilepsy. Ann Pharmacother. 2004;38:1631–4.

Talevi A, Bruno-Blanch LE. Efflux transporters at the blood–brain barrier: Therapeutic opportunities. In: Montenegro PA, Juárez SM, editors. Blood–brain barrier: new research. 1st ed. New York: Nova Science; 2012.

Talevi A, Sella-Cravero M, Castro EA, Bruno-Blanch LE. Discovery of anticonvulsant activity of abietic acid through application of linear discriminant analysis. Bioorg Med Chem Lett. 2007a;17:1684–90.

Talevi A, Bellera CL, Castro EA, Bruno-Blanch LE. A successful virtual screening application: prediction of anticonvulsant activity en MES test of widely-used pharmaceutical and food preservatives methylparaben and propylparaben. J Comput Aided Mol Des. 2007b;21:527–38.

Talevi A, Enrique AV, Bruno-Blanch LE. Anticonvulsant activity of artificial sweeteners: a structural link between sweet-taste receptor T1R3 and brain glutamate receptors. Bioorg Med Chem Lett. 2012;22:4072–4.

Thakur R, Gupta RB. Formation of phenytoin nanoparticles using rapid expansion of supercritical solution with solid cosolvent (RESS-SC) process. Int J Pharm. 2006;308:190–9. doi: 10.1016/j.ijpharm.2005.11.005.

Tishler DM, Weinberg KI, Hinton DR, Barbaro N, Annett GM, Raffel C. MDR1 gene expression in brain of patients with medically intractable epilepsy. Epilepsia. 1995;36:1–6.

Tiwari A, Sodani K, Dai CL, Ashby CR, Chen ZS. Revisiting the ABCs of multidrug resistance in cancer chemotherapy. Curr Pharm Biotechnol. 2011;12:570–94.

Trojnar MK, Wierzchowska-Cioch E, Krzyżanowski M, Jargiełło M, Czuczwar SJ. New generation of valproic acid. Pol J Pharmacol. 2004;56:283–8.

van Vliet EA, van Schaik R, Edelbroek PM, Redeker S, Aronica E, Wadman WJ, et al. Inhibition of the multidrug transporter P-glycoprotein improves seizure control in pehnytoin-treated chronic epileptic rats. Epilepsia. 2006;47:672–80.

Varshosaz J, Eskandari S, Tabakhian M. Production and optimization of valproic acid nanostructured lipid carriers by the Taguchi design. Pharm Dev Technol. 2010;15:89–96.

Vezzani A, Granata T. Brain inflammation in epilepsy: experimental and clinical evidence. Epilepsia. 2005;46:1724–43.

Vezzani A, Fench J, Bartfai T, Baram TZ. The role of inflammation in epilepsy. Nat Rev Neurol. 2011;7:31–40.

Volk HA, Arabadzisz D, Fritschy JM, Brandt C, Bethmann K, Löscher W. Antiepileptic drug resistant rats differ from drug responsive rats in hippocampal neurodegeneration and $GABA_A$ receptor ligand-binding in a model of temporal lobe epilepsy. Neurobiol Dis. 2006;21: 633–46.

Von Wedel-Parlow M, Wölte P, Galla HJ. Regulation of major efflux transporters under inflammatory conditions at the blood–brain barrier *in vitro*. J Neurochem. 2009;111:111–8.

Vreugdenhil M, Wadman WJ. Modulation of sodium currents in rat CA1 neurons by carbamazepine and valproate after kindling epileptogenesis. Epilepsia. 1999;40:1512–22.

Waldbaum S, Patel M. Mitochondrial dysfunction and oxidative stress: a contributing link to acquired epilepsy? J Bioenerg Biomembr. 2010a;42:449–55.

Waldbaum S, Patel M. Mitochondria, oxidative stress, and temporal lobe epilepsy. Epilepsy Res. 2010b;88:23–45.

Waldbaum S, Liang LP, Patel M. Persistent impairment of mitochondrial and tissue redox stratus during lithium–pilocarpine-induced apileptogenesis. J Neurochem. 2010;115:1172–82.

Whitaker WR, Faull RL, Dragunow M, Mee EW, Emson PC, Clare JJ. Changes in the mRNAs encoding voltage-gated sodium channel types II and III in human epileptic hippocampus. Neuroscience. 2001;106:275–85.

Williamson PR, Marson AG, Coffey AJ, Middleditch C, Rogers J, Bentley DR, et al. Clinical factors and ABCB1 polymorphisms in prediction of antiepileptic drug response: a prospective cohort study. Lancet Neurol. 2006;5:668–76.

Wohlfart S, Gelperina S, Kreuter J. Transport of drugs across the blood–brain barrier by nanoparticles. J Control Release. 2012;161:264–73.

Wong EH, Tarazi FI, Shahid M. The effectiveness of multitarget agents in schizophrenia and mood disorders: relevance of receptor signature to clinical action. Pharmacol Therapeut. 2010; 126:173–85.

Wu J, Ji H, Wang Y, Li YQ, Li WG, Long Y, et al. Glutathione depletion upregulates P-glycoprotein expression at the blood–brain barrier in rats. J Pharm Pharmacol. 2009;61:819–24.

Youdim MBH, Buccadfasco JJ. Multi-functional drugs for various CNS targets in the treatment of neurodegenerative disorders. Trends Pharmacol Sci. 2005;26:27–35.

Zeng TF, An DM, Li JM, Li YH, Chen L, Hong Z, et al. Evaluation of different antiepileptic drug strategies in medically refractory epilepsy patients following epilepsy surgery. Epilepsy Res. 2012;101:14–21.

Zhang P, Hu L, Wang Y, Wang J, Feng L, Li Y. Poly(ε-caprolactone)-blockpoly(ethyl ethylene phosphate) micelles for brain-targeting drug delivery: *in vitro* and *in vivo* valuation. Pharm Res. 2010;27:2657–69.

Zhang C, Kwan P, Zuo Z, Baum L. The transport of antiepileptic drugs by P-glycoprotein. Adv Drug Deliv Rev. 2012;64:930–42.