INTRINSIC SEVERITY AS A DETERMINANT OF ANTIEPILEPTIC DRUG REFRACTORINESS

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For the most part, resistance to medications in epilepsy is independent of the choice of antiepileptic drug. This simple clinical observation constrains the possible biological mechanisms for drug refractory epilepsy by imposing a requirement to explain resistance for a diverse set of chemical structures that act on an even more varied group of molecular targets. To date, research on antiepileptic drug refractoriness has been guided by the "drug transporter overexpression" and the "reduced drug-target sensitivity" hypotheses. These concepts posit that drug refractoriness is a condition separate from the underlying epilepsy. Inadequacies in both hypotheses mandate a fresh approach to the problem. In this article, we propose a novel approach that considers epilepsy pharmacoresistance in terms of intrinsic disease severity. We suggest that neurobiological factors that confer increased disease severity lead to drug intractability. The occurrence of frequent seizures at disease onset is an important factor that signals increased severity.

At the molecular level, marketed antiepileptic drugs (AEDs) reduce the incidence of seizures by effects on 1) voltage-gated sodium channels; 2) components of the GABA system including $GABA_A$ receptors, the GAT-1 GABA transporter and GABA transaminase; and 3) voltage-gated calcium channels (1). Recently, several additional molecular targets have been defined, including $\alpha_2\delta$, SV2A and Kv7/KCNQ/M potassium channels (2). Different AEDs acting on the same target may affect the target in biophysically distinct ways, and some AEDs act on more than one of the molecular targets. It is safe to say that no marketed AED acts in an identical fashion to any other, with the possible exception of carbamazepine and oxcarbazepine that may have very similar modes of action. There is no simple, universally accepted definition of drug refractory epilepsy (3). Operationally, however, we consider drug-resistant epilepsy to be epilepsy in which uncontrolled seizures persist despite state-of-the-art medical management. A neurobiological understanding of drug resistance in epilepsy requires an explanation of the failure to obtain seizure control despite the availability of nearly 25 different AEDs, each of which has distinct physical-chemical properties and modes of action.

Can Current Hypotheses Adequately Explain Epilepsy Pharmacoresistance?

The two prevailing hypotheses proposed to explain multidrug resistance in epilepsy are 1) the *transporter hypothesis*, which posits that there is inadequate access of AEDs to epileptic tissue because they are removed by multidrug transporters that are pathologically overexpressed, and 2) the *target hypothesis*, that proposes inherited or acquired alterations in the molecular targets of AEDs, leading to reduced pharmacodynamic effects of the drugs (4,5).

According to the drug transporter hypothesis, restricted access of AEDs to the seizure focus is the result of either locally increased expression of drug transporter proteins, most notably P-glycoprotein (P-gp, encoded by the *ABCB1* gene), or genetic variation in *ABCB1* resulting in increased transporter activity. Although there is a considerable body of evidence that is compatible with the transporter hypothesis, the proposed mechanism suffers from a lack of evidence that many clinically used AEDs are substrates for human P-gp or any other known human blood–brain barrier efflux transporter (6,7). Yet, for the hypothesis to truly explain clinical AED refractoriness, many if not all, marketed AEDs would need to be transported. The transporter hypothesis has also failed to receive support from recent genetic association studies including prospective analyses and a meta-analysis that failed to replicate early reports of an association between polymorphisms in the *ABCB1* gene and drug resistance (8–12). Moreover, the linear relationship between brain concentration and unbound serum concentration of AEDs, including in drug-resistant epilepsy patients, challenges the very existence of a saturable, protein-mediated AED transport system in humans (7). In sum, while there is evidence for increased transporter expression in seizure foci in animal models and in human tissue resected in epilepsy surgery, it has not yet been demonstrated that the transporter increases are

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functionally relevant and not simply an epiphenomenon of recurrent seizures or the underlying pathology.

According to the target hypothesis, epilepsy pharmacoresistance occurs when intrinsic (genetic) or acquired (disease related) changes in drug targets make them less sensitive to AEDs (4,5). Two recent studies have provided evidence of reduced sensitivity to carbamazepine in brain tissue from patients who were clinically unresponsive to carbamazepine and underwent resective surgery (13,14). Since the carbamazepine was applied directly to brain slices in these experiments circumventing the blood–brain barrier and any transporter effects, reduced sensitivity is presumably due to altered target sensitivity. However, both studies were restricted to carbamazepine only, and so it is unknown whether pharmacodynamic insensitivity in these tissues extended to AEDs with different mechanisms of action or even to other AEDs that target sodium channels. Since, as noted in our critique of the transporter hypothesis, patients with drug-resistant epilepsy are for the most part resistant to all AEDs, the validity of the target hypothesis is challenged by the broad range of molecular targets through which AEDs act, which would all need to be simultaneously modified to produce true multidrug resistance. The problem with this hypothesis is most starkly apparent in relation to the intrinsic (genetic) version of the target hypothesis, where all drug targets would have to be simultaneously present in their drug-insensitive forms in order to account for multidrug pharmacoresistance. Indeed, a polymorphism in the *SCN1A* gene encoding $Na_v1.1$ sodium channels has been described, which associates with the use of higher doses of carbamazepine and phenytoin, suggesting that there is reduced sensitivity to these drugs (8,9). However, this polymorphism is unlikely to influence sensitivity to AEDs that do not act through sodium channels and, since multidrug resistance would require the chance coincidence of multiple inherited drug resistance polymorphisms in multiple drug targets, it is an unlikely explanation for the refractory epilepsy phenotype.

The acquired version of the target hypothesis proposes that the pharmacodynamic sensitivity of the AED target is modified by the disease state. There are many examples of changes in the activity of voltage-gated and neurotransmitter-activated ion channels in acquired epilepsy models, some of which lead to reduced responsiveness to AEDs (5). For example, there is a loss in benzodiazepine sensitivity in a rat model of temporal lobe epilepsy resulting from alterations in the subunit composition of $GABA_A$ receptors, which are the molecular target of benzodiazepines (15). However, there is no evidence that the efficacy of AEDs acting on different targets are similarly affected, or even that other drugs (such as barbiturates) that act upon $GABA_A$ receptors but at a distinct site from benzodiazepines are affected. In fact, it seems unlikely that this would be the case given that the sensitivity of drugs that act via the benzodiazepine recognition site is critically dependent upon $GABA_A$ receptor subunit composition, whereas this is not true for other types of drugs that act on $GABA_A$ receptors.

In short, despite a decade of research, there is an absence of data sufficient to prove either the transporter or target hypotheses of multidrug resistance. Moreover, conceptual concerns suggest that the hypotheses may be leading us down the wrong path. We believe this mandates fresh thinking and the formulation of an alternative hypothesis capable of explaining the fundamental clinical observation that true drug resistance applies to a diverse group of unrelated chemical structures that act on a diverse set of molecular targets. The current view of multidrug resistance frames resistance as a problem isolated from the disease itself, and this has constrained research in the field, much in the way that cancer therapeutics would have been limited by a focus on mechanisms of drug resistance to the exclusion of the fundamentals of cancer biology. Indeed, despite an enormous effort to define a role for drug transporters including P-gp as a cause of multidrug resistance in cancer and to develop transporter-targeted pharmacological strategies to overcome drug resistance, this line of research has not impacted cancer survival. In contrast, certain therapies that take advantage of a deep understanding of cancer biology, such as the monoclonal antibody trastuzumab (Herceptin) or the tyrosine kinase inhibitor imatinib (Gleevec), have been remarkably successful (16). As far as we are aware, there is no evidence that epilepsy drug resistance evolves (or exists) separately from the epilepsy disease state itself but considerable evidence that the epilepsy in an individual patient has an inherent severity that defines the response to medication. If inherent severity is the decisive factor determining drug responsiveness, advances in the understanding and management of refractory epilepsy will first require an elaboration of the clinical criteria and biomarkers that define more severe forms of the disease that are associated with drug refractoriness. This will then empower investigations seeking to understand the pathophysiological basis of the severity. This is the traditional approach for diseases other than epilepsy, where new treatments have been developed without the need to first understand what makes the disease resistant to existing treatments.

Inherent Disease Severity as a Mechanism of Epilepsy Pharmacoresistance

Prospective studies of outcome in newly treated epilepsy have included population (17–19) and hospital-based cohorts (11,20–24), with all showing similar results. The studies demonstrate that rates of remission of seizures in newly diagnosed epilepsy have changed little in 20 years, despite the release of many new AEDs (25). Moreover, despite the heterogeneity of epilepsy etiology, a consistent finding across these studies is that the single most important factor associated with prognosis (the chance

of remission of seizures after diagnosis) is the frequency of seizures in the early phase of epilepsy, with an association between increased number of seizures in this period and poorer outcome (11,18,19,21). Both the number of seizures pretreatment (11,21,23) and in the immediate period after presentation (18) influence the chance of remission. Indeed, the frequency of seizures in the early phase of epilepsy is the dominant risk factor influencing the chance of remission of seizures, outweighing the contribution from other factors associated with prognosis including etiology of epilepsy, seizure type or the results of EEG or imaging investigations. In the National General Practice Study of Epilepsy (18), the effect of 4 seizures in the 6-month period after diagnosis of epilepsy compared to a single seizure was to reduce the chances of remission by approximately one-half, and the effect of 9 seizures reduced the chance of remission by two-thirds. In a hospital-based, "real-life" prospective cohort, patients with 11 or more seizures pre-treatment were more than twice as likely to be uncontrolled than patients with two or less seizures pretreatment, independent of the time from first seizure to starting treatment (21). These epidemiological data suggest that there are differences in inherent epilepsy severity reflected in the frequency of seizures in the early phase of epilepsy that influence an individual patient's response to medication, much in the same way that any other disease can vary from mild to severe and show a variable response to treatment. The observation that the occurrence of frequent seizures is associated with poorer outcome suggests that common neurobiological factors may underlie both epilepsy severity and drug refractoriness.

Are there factors that might undermine this interpretation of the epidemiological data? Where response to treatment is defined as achieving freedom from seizures for a given period of time, it has been suggested that there is a potential for infrequent seizures to inflate the estimate of therapeutic drug response (26). However, if infrequent seizures are as difficult to treat as frequent seizures, but give an erroneous impression of drug responsiveness because of the long interval of time between seizures, the association of seizure frequency with chance of remission should depend on the duration of the remission period analyzed. In fact, the association is the same whether remission of epilepsy is defined as absence of seizures for a period of 1 or 5 years duration (18). In addition, if patients with infrequent seizures and those with frequent seizures respond equally to medication, then the practice of empirical titration of AED dose according to seizure recurrence should result in patients with frequent seizures achieving more rapid titration and therefore achieving remission of seizures in a shorter period of time than patients with more widely spaced seizures. In fact, the opposite is observed, at least for the outcome of time to 12-month remission (11). An important alternative interpretation of the epidemiological data is that recurrent seizures render the epilepsy more resistant to treatment later on, leading to an acquired state of drug resistance. However, there is little evidence that "seizures beget seizures" in the vast majority of cases (25,27), and good evidence that the chances of long-term remission of seizures are not dependent on the duration of epilepsy or early drug therapy (21,22,24). Differential mortality among patients with varying degrees of epilepsy severity such that patients with more frequent seizures are followed for shorter periods because of death (deflating the estimate of chance of seizure remission) are recognized to have little impact on estimates of remission (18). However, epilepsy in the context of a developing brain may represent a special circumstance: some childhood epilepsies appear refractory before entering spontaneous remission and some relapse after early remission (19), and some childhood encephalopathies show progression over time. Similarly, epilepsy in the context of an evolving disease, and we might include mesial temporal sclerosis in this category, may become refractory after long periods of remission (28).

The concept that factors related to the occurrence of frequent seizures are associated with refractoriness seems biologically plausible: if the epilepsy is of a nature that seizures are easy to trigger leading to frequent seizures, then the seizures may also be more difficult to suppress. The observation in many acute seizure models that suppression of seizures conferred by any given dose of AED can be overcome by increasing the intensity of the pharmacological or electrical seizure stimulus (see e.g., ref. 29) suggests a simple physiological explanation of the epidemiological data: if susceptibility to seizures is sufficiently high, it may not be possible to prevent recurrence of seizures with any nontoxic dose of a currently available AED.

Whether genetic factors influence the frequency of seizures and therefore outcome is unknown. Surprisingly, no study has attempted to identify molecular genetic contributions to disease severity in epilepsy, and only a single study has attempted to measure the heritability of epilepsy outcome (30). In this study of 37 epilepsy concordant twin pairs (27 monozygotic, 10 dizygotic), no evidence for outcome specific genetic factors (such as that proposed for the drug transporter genes) was identified, although the study was small and a role for such factors cannot be discounted. The absence of studies addressing genetic contributions to severity of epilepsy likely reflects the absence of a meaningful definition of severity, as well as a dearth of costly and time consuming prospective genetic studies in epilepsy that carefully record the number of seizures pre- and posttreatment over time and which avoid sources of bias when measuring epilepsy outcome.

Conclusion

The inherent severity model of epilepsy proposes that there is a continuum in severity of the disease, which determines its relative response to medication. As yet poorly understood neurobiological factors account for disease severity. Increased frequency of seizures at the time of diagnosis is a signal of

increased severity and future drug refractoriness. However, clinical and experimental studies in epilepsy have generally ignored the concept of disease severity that is fundamental in the description of disease in other areas of medicine. The development of measures of epilepsy severity is urgently needed to enable clinical studies examining the prognostic implications of severity and its relationship to drug responsiveness. Such measures are also a prerequisite for studies of the neurobiological factors that underlie disease severity, which will be critical to the development of strategies to overcome pharmacoresistance.

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