Mechanisms of action of antiepileptic drugs: the search for synergy

Carl E. Stafstrom

Pediatric Neurology Section, Department of Neurology, University of Wisconsin, Madison, Wisconsin, USA

Correspondence to Carl E. Stafstrom, MD, PhD, Department of Neurology, 600 Highland Avenue, H6-528, University of Wisconsin, Madison, WI 53792, USA
Tel: +1 608 262 2154; fax: +1 608 263 0412; e-mail: stafstrom@neurology.wisc.edu

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Purpose of review

The aim is to review rational polytherapy of antiepileptic drugs in terms of conventional and novel mechanisms of action, consider combinations that might be beneficial when used as polytherapy, and discuss whether animal models can predict clinical efficacy.

Recent findings

Many patients with epilepsy require concurrent treatment with more than one antiepileptic drug (rational polytherapy), but there is little information available as to which drugs might work best in combination. Conventional antiepileptic drugs act by blocking sodium channels or enhancing γ-aminobutyric acid function. Some newer antiepileptic drugs have novel mechanisms of action, including impairment of the slow inactivation of sodium channels, binding to the presynaptic vesicle protein SV2A, binding to the calcium channel α2δ subunit, and opening select potassium channels. Several antiepileptic drugs have multiple or uncertain mechanisms of action. Quantitative techniques such as isobolography can be used to compare the efficacy and side effects of antiepileptic drug combinations in animals. However, neither such methods nor antiepileptic drug mechanisms of action have yet proven useful in predicting clinical benefit in patients.

Summary

Animal models can be used to help predict drug combinations that might be effective clinically, based on novel mechanisms of action. However, at this point, antiepileptic drug choice in patients with epilepsy remains empirical.

Keywords

antiepileptic drugs, epilepsy, isobolography, mechanisms of action, seizures, synergy

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Introduction

Treatment with antiepileptic drugs (AEDs) is the foundation of epilepsy therapeutics. A patient with epilepsy is started on monotherapy with a drug chosen to act in accordance with the main seizure type, also taking into consideration the drug's mechanism of action and side effect profile as well as relevant patient characteristics such as age, comorbidities, and concurrent medical treatments. About 1/2 to 2/3 of patients respond initially to AED monotherapy; approximately 47% respond to the initial AED, and if that drug is not effective, substitution of a second AED as monotherapy may benefit another 13% of patients [1]. If those two drugs do not bring about seizure control, the chance of further benefit with additional monotherapy trials rapidly drops off to even smaller percentages. The remaining patients require treatment with more than one AED [2,3•]. Whereas monotherapy is preferable and results in fewer adverse side effects, it is an unfortunate reality of clinical practice that many patients are treated with two or more AEDs simultaneously (polytherapy). However, there is minimal clinical data on effective AED polytherapy combinations, making optimal treatment of these refractory patients a major challenge for neurologists.

Rational polytherapy refers to the intentional choice of the second AED to enhance seizure control [4]. Rational polytherapy presupposes that two AEDs with different mechanisms of action may provide better seizure control than two drugs with a similar mechanism. But which drug combinations would most likely yield improved seizure control without undue adverse effects? In a patient on monotherapy, when a second drug is added, three possibilities exist: the clinical effects of the two drugs could be simply the sum of the effect of each drug alone (additive), the effect of adding the second drug could exceed the individual effect of each drug alone (synergistic or supra-additive), or the effect of the two drugs together could be less than the effect of each drug alone (antagonistic or infra-additive). Similarly, the adverse effects or toxicity of a drug combination can be additive, synergistic, or antagonistic. Regarding clinical effectiveness, synergy (or, at least, additivity) is desirable; for

adverse effects, antagonism is preferable. The therapeutic aim of AEDs is to achieve the lofty goal of 'no seizures, no side effects'. For practical purposes, it might be acceptable to utilize a drug combination that exhibits intermediate effects, such as two drugs that are additive with regard to their clinical effect but antagonistic with regard to their side effects, or two drugs that are synergistic clinically but their side effects are only additive.

The potential combinations of AEDs and doses are almost limitless; to determine optimal drugs or drug combinations to treat seizures, a systematic, quantitative approach is warranted. Here, I briefly review recent information on the mechanisms of action of AEDs, focusing on some of the newer AEDs that have not yet been used extensively in the clinical arena, especially as polytherapy. Next, the use of animal models to screen AEDs is described as a potential method by which to identify efficacious drug combinations. Finally, human data are compared with data generated from animal models in an attempt to provide a practical approach to combination AED therapies.

Table 1 Antiepileptic drugs categorized by mechanism of action

Sodium channel blockers
Phenytoin
Carbamazepine
Oxcarbazepine
Lamotrigine
Zonisamide
Lacosamide
Rufinamide
Calcium channel blockers
Topiramate
Lamotrigine
Ethosuximide
GABA enhancers
Benzodiazepines
Tiagabine
Vigabatrin
Phenobarbital
Glutamate receptor antagonists
Topiramate
Felbamate
Multiple mechanisms
Valproic acid
Topiramate
Felbamate
Phenobarbital
Gabapentenoids
Gabapentin
Pregabalin
Potassium channel openers
Retigabine
Diuretics
Bumetanide
Acetazolamide
Other
Levetiracetam
Stiripentol

Mechanisms of action of antiepileptic drugs: 'something old, something new'

Numerous reviews of anticonvulsant mechanisms of action are available, both for the so-called older or conventional AEDs as well as for the newer generations of AEDs introduced since the 1990s [5–9]. Several new AEDs with novel and unexpected mechanisms of action have been approved recently or will be soon. AED mechanisms are typically defined through in-vitro experimental studies of ion channels, synaptic transmission, neurotransmitter uptake and metabolism, and so on (Table 1). Knowledge of conventional and novel AED mechanisms of action offers the opportunity to design a polytherapy regimen that is truly rational [10].

Many of the older AEDs act by blocking excitatory current through sodium or calcium channels [11]. Sodium currents underlie the normal and repetitive firing behavior of neurons. The fast, transient sodium current gives rise to action potentials, whereas a persistent sodium current modulates subthreshold excitability [12]. After opening in response to membrane depolarization, sodium channels inactivate in two kinetic phases: fast and slow. Sodium channel-blocking AEDs such as phenytoin and carbamazepine bind to the active state of the channel and reduce high frequency firing (as might occur during a seizure) while allowing normal action potentials to occur. Similarly, some of the newer AEDs such as oxcarbazepine, lamotrigine, and zonisamide act by facilitating the fast inactivation of sodium channels [6]. Rufinamide, a new AED with broad-spectrum action against many seizure types that has promising effects in multiple seizure types including Lennox-Gastaut syndrome, also appears to increase fast inactivation [13,14]. Lacosamide, however, is unique in selectively enhancing only the slow form of sodium channel inactivation, with no effect on fast sodium channel inactivation, GABA receptors, potassium channels or calcium channels [15•,16]. Slow inactivation involves modification of the shape of the sodium channel and occurs over the time course of seconds to minutes and could ameliorate prolonged neuronal firing as might occur in a seizure; fast inactivation occurs on a milliseconds time scale. Furthermore, preliminary evidence links lacosamide to modulation of the collapsin-response mediator protein-2, which is involved in neuronal growth and plasticity [16].

Calcium channels mediate multiple cellular effects including fusion of neurotransmitter-containing vesicles with the presynaptic terminal membrane, thus allowing release of neurotransmitter. Some calcium channel-blocking AEDs are effective against partial and generalized tonic–clonic seizures. Lamotrigine blocks high voltage activated (HVA) calcium channels and zonisamide has action at T-type calcium channels [17], with lamotrigine having an additional effect on absence seizures and other seizure types. Ethosuximide, the prototypical

AED for absence seizures, blocks T-type calcium channels in thalamic neurons [18] and may also decrease the persistent sodium current [19].

Agents that enhance GABA action are generally thought to suppress seizures by increasing neuronal inhibition. Multiple molecular mechanisms lead to enhanced GABA action, including an increase in the open time (barbiturates) or opening frequency (benzodiazepines) of the GABA-A receptor-mediated chloride channel. Recent data suggest that phenobarbital has additional actions on HVA channels and glutamate receptors [6], placing it into the category of a multifunctional AED (discussed in the next paragraph). Other GABA enhancers have specific effects and in fact were 'designed' to increase inhibition – tiagabine blocks the presynaptic and astrocytic GABA transporter and vigabatrin inhibits GABA transaminase, the enzyme responsible for breaking down GABA. Although both drugs increase GABA availability, their mechanisms of action are likely more complex such as increasing tonic GABAergic inhibition [20].

Interestingly, other drugs designed to potentiate GABAergic activity turn out to have mixed or complex mechanisms of action. The primary molecular target for pregabalin and gabapentin appears to be the α2δ subunit of voltage-activated calcium channels [8,21•]. Binding to this site reduces release of neurotransmitter, especially glutamate.

Levetiracetam binds to the synaptic vesicle protein SV2A, decreasing calcium influx into the presynaptic terminal [22]. It is unclear how levetiracetam suppresses seizures, perhaps related to decreased release of excitatory neurotransmitter [23•]. Levetiracetam also impedes the development of kindling [24], suggesting that it can retard epileptogenesis as well as suppress seizures. However, given the action of levetiracetam against kindling, it is not clear whether the delay in acquisition is the result of its anticonvulsant effect or an ability to modify the epileptogenic process. Adding to its multifunctional effects, levetiracetam also blocks HVA calcium channels and alters GABA function.

Continuing the theme that many currently used AEDs have mixed, complex, or poorly understood mechanisms of action, valproate increases GABA turnover and elevates brain GABA levels, alters some types of potassium and calcium channels, and modulates fast and persistent sodium channels [6]. The broad antiepileptic clinical spectrum of valproate attests to the multiple mechanisms underlying its function. Similarly, topiramate has multiple mechanisms of action including reduction of repetitive firing via sodium channel blockade and enhancement of GABA activity [7]. Topiramate also has actions against kainate and α-amino-3-hydroxyl-5-methyl-4-isoxazole-proprionate (AMPA)-type glutamate receptors and decreases neuronal excitation. Felbamate, another broad-spectrum agent, is especially useful for treatment of the multiple seizure types seen in Lennox-Gastaut syndrome as well as partial, generalized and perhaps absence seizures. Felbamate has multiple targets of action including fast sodium channels, HVA calcium channels, GABA receptors and *N*-methyl-D-aspartate (NMDA)-type glutamate receptors [7].

Many newer agents have unique and interesting mechanisms of action. Stiripentol increases GABA release, inhibits GABA uptake and directly modulates some GABA-A receptor subtypes [25,26•]; these mechanisms are of considerable interest since stiripentol shows promising effectiveness in Dravet syndrome, which is caused by mutations in the sodium channel *SCN1A* gene [27,28]. Retigabine and its congeners operate as openers of the potassium channel subtype, Kv7, enhancing M-type potassium current and reducing neuronal excitability [29,30].

A promising therapy for neonatal seizures is bumetanide, a loop diuretic similar to furosemide. In neonatal neurons, the chloride (Cl^-) gradient across the cell membrane is reversed from the adult situation, with a higher Cl^- concentration inside the cell. Therefore, GABA binding to its receptor opens the Cl^- channel and Cl^- rushes to the outside of the cell, down its concentration gradient, causing depolarization of the neuronal membrane. The Cl^- gradient in immature neurons is maintained by the sodium–potassium–chloride transporter, NKCC1. The expression of this transporter decreases over the first few weeks of life, after which the intracellular chloride concentration is lower than the extracellular concentration, and GABA binding causes hyperpolarization (the usual situation in mature neurons). Bumetanide inhibits NKCC1, such that GABA binding leads to inhibition rather than excitation, mimicking the mature case. Bumetanide has been found to abate epileptiform activity both *in vitro* and *in vivo* [31••,32•].

Clinical effects of multiple antiepileptic drug combinations: 'can 1 + 1 >2?'

Given the wide array of therapeutic options now available, even the choice of monotherapy can be daunting. When two or more drugs are used together, the situation becomes even more complex. In addition to mechanistic issues, drug interactions must be considered. This review focuses on pharmacodynamic interactions and does not discuss pharmacokinetic interactions.

Clinical data legitimizing rational polytherapy are quite limited. Even when polytherapy is chosen to reflect specific mechanisms of action, the desired clinical response may not follow. The question arises whether

it is better to use two drugs with different mechanisms of action, or two drugs with a similar mechanism of action? In the first case, the complementary mechanisms of two AEDs may reduce seizures by a two-pronged approach. For example, two sodium channel blockers may be less effective than a sodium channel blocker in combination with a GABA agonist. The second possibility might be conceptualized as a more complete attack on a single cellular mechanism; for example, the combination of an AED that increases fast inactivation of the sodium channel (e.g. phenytoin, carbamazepine) with an AED that boosts slow inactivation of the sodium channel (e.g. lacosamide) could, at least theoretically, yield synergy though clinical data are lacking.

An equally if not more important issue in polytherapy is to minimize adverse effects. For instance, concurrent use of two benzodiazepines would likely lead to increased sedation, and the combination of carbamazepine and oxcarbazepine would probably cause unacceptable dizziness. It might be possible to use lower doses of each medication rather than raising monotherapy to its maximal tolerated dose [4]. The concurrent use of valproate and ethosuximide for absence seizures is one such example.

Some clinical data suggest that specific combinations of AEDs are synergistic, for example, the concurrent use of valproate and lamotrigine [33]. In a study of 1617 patients who were seizure-free for more than 1 year, 21% were taking more than one AED; the most efficacious combinations were lamotrigine and valproate, phenytoin and phenobarbital, carbamazepine and gabapentin, and carbamazepine and valproate [34].

To perform clinical trials to quantitatively evaluate the huge number of possible combinations of doses and medications in patient populations is quite difficult [35]. Furthermore, AED mechanisms of action, as described above, are probably more complicated than previously thought and surprising mechanistic combinations might emerge. Most clinical trials of AEDs are add-on, thereby comprising, by definition, clinical polytherapy experiments. Currently, there is no routine way to systematically evaluate and identify preferred combinations of AEDs from clinical trial data or postmarketing exposure.

Animal models: 'better living through isobolography?'

Animal studies allow assessment of a much larger range of doses and dose combinations than do clinical studies. Over many decades, AED screening programs have tested the effects of thousands of compounds for their potential seizure-suppressant effects [36]. Two main animal models are used for high-throughput screening of potential AEDs. Maximal electric shock (MES) is an accepted model for generalized tonic–clonic and partial-onset seizures. The second animal model uses subcutaneous pentylenetetrazole (PTZ), a GABA-A receptor antagonist, as a paradigm for myoclonic seizures and overall seizure susceptibility. Recently, alternative models have been developed to evaluate other seizure types. The 6-Hz corneal stimulation model is used to assess limbic seizures and may be informative as a model of pharmacoresistant epilepsy. For example, phenytoin has no effect on 6-Hz seizures and levetiracetam is ineffective in MES and PTZ but effective in the 6-Hz model [37,38]. In all models, clinical trial data are needed to confirm preclinical results.

In addition to acute anticonvulsant screens, there is increasing recognition of the need for drugs to retard the process of epileptogenesis. Kindling is one well established method to study the effect of AEDs on epileptogenesis [39,40]. In kindling, initially subconvulsive stimuli to various brain regions result, after many days of stimulation, in limbic seizures that secondarily generalize. Other chronic epilepsy models, both acquired and genetic, need to be incorporated into AED/polytherapy testing [41]. Disadvantages implicit in current animal models include the almost exclusive use of adult rats, usually males, and nearly always using drug-naïve animals with normal neurologic function (i.e. rats that are not epileptic).

Typically, drugs are characterized by their ED50 or EC50 (the effective dose or serum concentration, respectively, at which 50% of animals are protected from a particular seizure stage). To test drug combinations, the EC50 or ED50 of one drug is determined; the effect of adding a second drug on the original drug's ED50 or EC50 is then assessed. Quantitative methods such as isobolography have long been applied to study drug combinations [42]. In isobolography, the effects of two drugs in various combinations of doses are compared to determine whether the potency or toxic effect of one drug is synergistic, additive, or antagonistic to a second drug [43–45]. In the same way, side effect endpoints can be measured. Using isobolography, a large number of potentially beneficial AED combinations have been identified, as well as some interactions that are potentially disadvantageous. A recent review summarizes isobolographic studies of AEDs in animal models [46••]. Table 2 lists combinations shown to be additive or synergistic in isobolographic studies. The fact that the majority of isobolographic studies come from a single laboratory lends consistency to the data but also emphasizes a need for independent replication. The choice of seizure model is critical for interpretation of isobolographic data.

Table 2 Possible synergistic antiepileptic drug combinations (based on preclinical data, mainly from studies using isobolography)

VPA + LTG[a]
GPN + VGB[a]
OXC + LEV[a]
OXC + GPN[a]
OXC + TGB[a]
LEV + TPM[a]
LEV + CBZ[a]
LTG + TPM[a]
TGB + GPN[a]
VPA + PHT
VPA + ESX
VPA + GPN
VPA + TPM
VPA + VGB
CBZ + GPN
CBZ + TPM
OXC + TPM
PHB + TPM
TPM + FBM

CBZ, carbamazepine; ESX, ethosuximide; FBM, felbamate; GPN, gabapentin; LEV, levetiracetam; LTG, lamotrigine; OXC, oxcarbazepine; PHB, phenobarbital; PHT, phenytoin; TGB, tiagabine; TPM, topiramate; VGB, vigabatrin; VPA, valproic acid.
[a] Combinations considered particularly promising based on synergistic effectiveness and favorable or antagonistic toxicity profile.

Perusal of this long list of AED combinations in Table 2 raises several questions. Is there a rank order of efficacy in preclinical studies that can be translated to patient care? Is there any mechanistic consistency or sense to the large number of synergistic AED combinations? Are any combinations ineffective or antagonistic? One trend is that combinations that include valproate, levetiracetam, and carbamazepine or oxcarbazepine appear to be particularly effective in seizure models. Gabapentin and topiramate also appear frequently on the list. Lamotrigine with topiramate or valproate demonstrate the additional promise of having antagonistic adverse effects or no neurotoxicity on behavioral testing [46••]. These combinations are also useful clinically, though there is no underlying common mechanism. That is, prediction of clinical efficacy is not obvious based on isobolographic analyses.

Recent studies expand the isobolographic method to other AEDs and seizure models. In the 6-Hz model, levetiracetam combined with phenobarbital offered synergistic protection, whereas levetiracetam combined with several other AEDs afforded additive efficacy [47•]. Building upon the emerging efficacy of loop diuretics as anticonvulsants, as described above for neonatal seizures, recent work shows that ethacrynic acid halved the ED50 of topiramate in the MES test [48]. An exciting potential combination of AEDs for neonatal seizures, which makes mechanistic sense, has been shown to reduce GABA-mediated excitation with bumetanide and use concurrent phenobarbital to enhance GABA-mediated inhibition [31••,49]. Other potential AED combinations could combine blockade of neuronal excitation with potentiation of inhibition, for example, an AMPA antagonist and a sodium channel blocker, or a GABAergic agent with an AMPA or NMDA-receptor antagonist. There is extensive investigation into optimizing glutamate receptor antagonists for use in epilepsy and other neurologic disorders [50].

The interactions of drugs with dietary treatments remain relatively unexplored. Acetone, a ketone body with anticonvulsant properties that is elevated during ketogenic diet administration, enhances the beneficial effects of valproate, carbamazepine, lamotrigine, and phenobarbital in MES [51•]. In an uncontrolled clinical study of children on the ketogenic diet, concurrent zonisamide was significantly more likely, and concurrent phenobarbital less likely, to produce more than 50% seizure reduction [52].

Whereas many synergistic AED combinations have been identified by isobolography, antagonism has also been found. The combination of lamotrigine and oxcarbazepine or carbamazepine yielded poorer seizure control than would be predicted, suggesting that these two agents might not be beneficial clinically [53]. However, many clinicians have used this combination successfully. Therefore, despite the large volume of experimental work, it remains uncertain whether isobolographic studies have direct clinical relevance. It has been concluded that no combination of drugs, predicts efficacy regardless of drug mechanisms of action [4,45,46••,54].

To determine cognitive changes or quantify the degree of toxicity in animal studies is also challenging. Most high-throughput studies can realistically assess only gross measures of neurologic function. The chimney test, frequently used in isobolographic studies to assess motor function, requires an animal to climb backwards up the inside of a plastic tube [53]. Subtler motor, memory, and cognitive changes cannot be easily assessed in animals, especially in high-throughput studies, although attempts are being made to expand the behavioral testing repertoire [47•,55]. In animals, AED adverse effects such as ataxia, sedation or impairment of motor function can be measured to an extent, but more subjective adverse effects such as dizziness or nausea cannot. Any conclusion about behavioral effects must be interpreted in light of the serum concentration of the drug.

A major limitation of isobolography, despite its elegant quantitative design, is the performance of these tests on normal (nonepileptic) rats. It is becoming increasingly clear that normal and epileptic patients can differ markedly in their response to AEDs, speaking to the need for innovative models and paradigms [56,57]. Similarly, the effects of AED combinations at different ages are largely

unexplored. Animals used in experimental studies are also pharmacologically naïve, whereas patients with epilepsy in clinical trials have typically been exposed to numerous AEDs. Finally, long-term pharmacodynamic effects beyond the acute experiments could turn out to be important, as might the influence of pharmacogenomic factors [58].

Conclusion

The profusion of new AEDs in the past several years has expanded the therapeutic arsenal and improved pharmacological treatment of epilepsy. Nevertheless, the field of drug interactions is still in its infancy and the choice of AED combinations for a given patient remains largely empirical. Clinicians faced with therapeutic decisions with regard to second or third AEDs have little data by which to guide decisions. Theoretical considerations based on AED mechanisms of action have not proven to be particularly informative, at least at this stage. Studies of AED synergy using animal models, including isobolographic techniques, have produced an enormous amount of data, with uncertain clinical applicability. The variety of seizure types in different epilepsy syndromes may or may not respond to drug combinations identified as effective by animal models, but the emergence of novel AED mechanisms of action offer renewed opportunities to discover synergistic interactions. A multicenter database would provide extensive clinical data to compare AED combinations.

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