Issues related to development of new antiseizure treatments

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SUMMARY

This report represents a summary of the discussions led by the antiseizure treatment working group of the International League Against Epilepsy (ILAE)/American Epilepsy Society (AES) Working Groups joint meeting in London (London Meeting). We review here what is currently known about the pharmacologic characteristics of current models of refractory seizures, both for adult and pediatric epilepsy. In addition, we address how the National Institute of Neurological Disorders and Stroke (NINDS)-funded Anticonvulsant Screening Program (ASP) is evolving to incorporate appropriate animal models in the search for molecules that might be sufficiently novel to warrant further pharmacologic development. We also briefly address what we believe is necessary, going forward, to achieve the goal of stopping seizures in all patients, with a call to arms for funding agencies, the pharmaceutical industry, and basic researchers.

KEY WORDS: Antiseizure drug, Pharmacoresistant epilepsy, Animal models of epilepsy.

Despite the development and availability of >22 antiseizure drugs (ASDs), most of which have been identified as efficacious based on large, double-blind, randomized clinical trials, it is estimated that at least 25–40% of newly diagnosed epilepsy patients will remain resistant to drug therapy and continue to have seizures (Schmidt & Sillanpaa, 2012). These continued seizures have considerable impact on the patients’ quality of life and greatly increase the risks of injury, socioeconomic disadvantage, and even death. Continuing seizures can also interfere with memory, cognitive function, and educational opportunities, and they may produce ongoing endocrine dysfunction (England et al., 2012). Therefore, the need for more effective therapies remains urgent. However, because the marketplace is already awash with ASDs, many pharmaceutical companies now refrain from the expensive enterprise of developing new compounds. Therefore, the ability of the epilepsy research community to convince a limited number of pharmaceutical and biotechnology companies to finance the development of promising new compounds is a growing concern. This article summarizes the discussions that took place at the London Workshop to address the strategies of how best to develop new therapies for patients who are resistant, or refractory, to...
existing ASDs and how to engage a pharmaceutical industry with limited resources to participate.

Most ASDs have been developed with the primary goal of stopping seizures, and current drug discovery is based on screening in animal models of acute seizures and epilepsy. Given the failure of new ASDs that help in controlling the refractory population to arise out of testing in these existing models, the field is now engaged in developing new models for preclinical testing. Although development in this area has been moving forward with new animal models of drug-resistant seizures, the efficacy of the limited number of ASDs that have been successful in those models is yet to be reflected clinically (Szoeke & Sillanpaa, 2012). Therefore, there was general agreement at the London meeting that there remains an urgent and continuing need to develop, pharmacologically characterize, and validate animal models that mimic the various human epilepsies and can therefore predict efficacy in patients with drug-resistant seizures.

To aid discussions of this critical topic, definitions of drug resistance have been formulated. These definitions are a result of a consensus obtained at a workshop held at the National Institutes of Health (NIH) in 2002 (Stables et al., 2003), by the International League Against Epilepsy (ILAE) (Kwan et al., 2010), and by the authors and participants of the 2012 London Workshop. Drug resistance in humans is herein described as the failure of a patient’s seizures to respond to at least two antiseizure medications that are appropriately chosen, used for an adequate period, and taken with proper adherence to the prescribed regimen. A patient may experience either complete drug resistance or, if seizures are reduced in either frequency and/or intensity, a patient may be classified as exhibiting partial responsiveness. Likewise, drug resistance in animal models is defined as persistent seizure activity that does not respond to monotherapy with at least two appropriate ASDs. In addition, in vitro drug resistance is defined as persistent epileptiform activity that correlates with seizures and is not abated by at least two appropriately chosen ASDs. The underlying mechanisms of pharmacoresistance in epilepsy remain mysterious, with several hypotheses proposed but not proven (Schmidt & Loscher, 2009). It is likely that genetic variability makes a significant contribution and ongoing advances in the pharmacogenomics field should improve our understanding of drug-resistant epilepsy, and help to identify novel targets for therapeutic intervention, and may ultimately lead to personalized prescribing of both existing ASDs and novel agents that optimizes therapy for the individual patient (Szoeke et al., 2006).

The workshop goal was to elucidate strategies for the identification and development of novel therapies within the constraints of current knowledge. New drugs for patients with refractory epilepsy might be more easily identified if we understood why some patients respond well to ASDs while others do not. Sadly, our current level of understanding of the basic mechanisms underlying epilepsy and drug responsiveness is lacking. However, we hypothesize that progress can be made toward obtaining novel therapies with the use of relevant animal models. Therefore, we review here what is currently known about the pharmacologic characteristics of current models of refractory seizures, both for adult and pediatric epilepsy. In addition, we address how the National Institute of Neurological Disorders and Stroke (NINDS)-funded Anticonvulsant Screening Program (ASP) is evolving to incorporate newer animal models in the search for molecules that might be sufficiently novel to warrant further pharmacologic development. We also briefly address what we believe is necessary, going forward, to achieve the goal of stopping seizures in all patients, with a call to arms for funding agencies and basic researchers.

**Models of Drug-Resistant Adult Epilepsy**

Many animal models of drug-resistant epilepsy have been developed over the last several decades. These provide a platform for understanding the basic mechanisms of pharmacoresistance and an opportunity to use more clinically relevant models of chronic epilepsy in drug discovery. However, surprisingly little is known about their pharmacologic profiles. This lack of information regarding pharmacologic sensitivity may be an impediment to future drug discovery efforts, as it is not yet clear which animal models are optimal for screening of compounds. London meeting participants expressed concern that it is becoming increasingly difficult to convince funding agencies of the need for such characterization. Another important consideration is the length of time it would currently take to validate the utility of any given model (i.e., demonstrating the efficacy of compounds selected by the model in refractory epilepsy patients). Nevertheless, in this section, we summarize what is currently known about the pharmacologic profiles of the more commonly used animal models of drug-resistant epilepsy to help determine if these models share drug response phenotypes with the seizure disorders they supposedly recapitulate. We anticipate that this summary may be useful for the research community in determining where efforts should focus when attempting to identify the most robust animal models for future drug discovery.

**In vivo models of drug-resistant acute seizures and chronic epilepsy**

A diverse group of animal models of acquired and genetic epilepsies now exists. The challenge now is to characterize the efficacy of existing ASDs in these models and to publish that data in a clear and consistent manner.
even where it is negative. Table 1 summarizes the effects of currently licensed ASDs in the more commonly used models of acquired epilepsy or drug-resistant acute seizures. Two specific cautions apply—doses used in animal models may result in concentrations that do not reflect therapeutic levels in humans and differences between specific strains of animals are not considered.

6-Hz Stimulation Model

When the maximal electroshock seizure (MES) test failed to identify levetiracetam (LEV) as a potential ASD, the ASP resurrected the 6-Hz model in an effort to detect potentially useful ASDs that might otherwise have been overlooked (Brown et al., 1953). Seizures are induced by electrical stimulation of the cornea at a frequency of 6 Hz and are characterized by an initial stun, followed by forelimb clonus, twitching of the vibrissae, and finally a Straub-tail (Barton et al., 2001). At low stimulus intensities there is little discrimination between ASDs, with all compounds tested conferring protection against seizures (Barton et al., 2001). However, at a stimulus intensity of 32 mA, it was no longer possible to determine an effective dose which protects 50% (ED$_{50}$) of the animals for either phenytoin (PHT) or lamotrigine (LTG). Finally, at the highest stimulus intensity used (44 mA), only valproic acid (VPA) and LEV were able to prevent seizures, albeit with higher ED$_{50}$ (Barton et al., 2001). The ASP and others now routinely employ the 6-Hz test for screening due to the relative insensitivity of this test to standard ASDs (Gasior et al., 2010; Byrtus et al., 2011; Kaminski et al., 2011). The rationale for this is that efficacy in the 6-Hz test may be indicative of a mechanism of action that is sufficiently unique to encourage the sponsor of the respective compound to continue development.

Kindling models

The amygdala and hippocampal kindling models are proposed models of temporal lobe epilepsy (TLE) and both are widely utilized in ASD screening efforts. Validation of these models for therapy discovery has occurred, with protection against fully kindled seizures often predictive of efficacy in the symptomatic treatment of partial seizures in patients. For the most part, these models are relatively sensitive to existing ASDs, although a lack of efficacy has been reported with some compounds, for example, topiramate (TPM). Therefore, it is arguable whether standard kindling models could be considered models of drug-resistant epilepsy. Several groups have, however, modified and further developed the traditional kindling protocol, leading to greater therapy resistance. Work from Löschner’s group has identified two distinct populations of kindled Wistar rats: one with fully kindled

Table 1. Pharmacologic properties of animal models of acute and chronic drug-resistant seizures

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Sensitivity to Spontaneous Seizures</th>
<th>Resistant to Spontaneous Seizures</th>
<th>Seizure type</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-Hz acute seizures (44 mA)</td>
<td>EZG, LEV, VPA</td>
<td>PHT, TPM, LTG</td>
<td>Complex partial seizures</td>
<td>Barton et al., 2001</td>
</tr>
<tr>
<td>Corneal kindled mouse</td>
<td>EZG, LEV, LTG, PHT, TGB, CBZ, VGB, VPA</td>
<td>TPM</td>
<td>PM</td>
<td>Rowley &amp; White, 2010</td>
</tr>
<tr>
<td>Amygdala kindled rat</td>
<td>VPA, FBM, CZP, GBP, LTG, LEV</td>
<td>TPM</td>
<td>Focal seizures that secondarily generalize</td>
<td></td>
</tr>
<tr>
<td>LTG-resistant kindled rat</td>
<td>FBM, EZG, VPA</td>
<td>LTG, CBZ, PHT, TPM</td>
<td>Focal seizures that secondarily generalize</td>
<td>Postma et al., 2000; Srivastava &amp; White, 2013</td>
</tr>
<tr>
<td>PHT-resistant kindled rat</td>
<td>LEV, LTG, FBM, GBP, TPM</td>
<td>PHT, VGB</td>
<td>Focal seizures that secondarily generalize</td>
<td>Loscher et al., 1993, 2000; Ebert et al., 1999, 2000</td>
</tr>
<tr>
<td>KA-induced SRS</td>
<td>CBZ</td>
<td>TPM* (seizure freq reduced by 56%)</td>
<td>Focal seizures that secondarily generalize</td>
<td>Grabenstatter et al., 2005, 2007</td>
</tr>
<tr>
<td>PILO-induced SRS</td>
<td>LEV, PB, CBZ, PHT</td>
<td>ESM</td>
<td>Focal seizures that secondarily generalize</td>
<td>Leite &amp; Cavaleiro, 1995; Glien et al., 2002</td>
</tr>
<tr>
<td>PB-resistant PILO-induced SRS</td>
<td>PB, PHT</td>
<td>Focal seizures that secondarily generalize</td>
<td>Bankstahl et al., 2012</td>
<td></td>
</tr>
<tr>
<td>Intrahippocampal KA (mouse)</td>
<td>PGB, LEV, VGB, DZP, TGB</td>
<td>VPA, LTG, PHT, CBZ</td>
<td>Focal seizures</td>
<td>Riban et al., 2002; Langlois et al., 2012</td>
</tr>
<tr>
<td>Intrahippocampal KA (rat)</td>
<td>PB</td>
<td>VB, PHT</td>
<td>Focal seizures</td>
<td>Rattka et al., 2013</td>
</tr>
<tr>
<td>Electriically induced SRS</td>
<td>VPA, LTG, VGB, CZP</td>
<td>CBZ, carisbamate</td>
<td>Focal seizures</td>
<td>Bethmann et al., 2007</td>
</tr>
<tr>
<td>TBI</td>
<td>CBZ, carisbamate</td>
<td>CBZ, VPA</td>
<td>Spreading seizures</td>
<td>Eastman et al., 2011</td>
</tr>
</tbody>
</table>

ASD, antiseizure drug; SRS, spontaneous recurrent seizures; KA, kainic acid; PILO, pilocarpine; TBI, traumatic brain injury; EZG, ezogabine; LTG, lamotrigine; CBZ, carbamazepine; VPA, valproic acid; LEV, levetiracetam; TPM, topiramate; TGB, tiagabine; PHT, phenytoin; PB, phenobarbital; FBM, felbamate; PGB, pregabalin; DZP, diazepam; CZP, clonazepam; VGB, vigabatrin; GBP, gabapentin; ESM, ethosuximide; *unpublished ASP data; †effective for only 1 week.
seizures that are suppressed by PHT and the other in which seizures are resistant to PHT. When the PHT-resistant subgroup of kindled animals is profiled further (Table 1), it shows differential responsiveness to other ASDs, with sensitivity to the antiseizure effects of LEV, LTG, felbamate (FBM), and gabapentin (GBP), and resistance to vigabatrin (VGB) (Loscher et al., 1993, 2000; Cramer et al., 1998; Ebert et al., 2000; Reissmuller et al., 2000).

A LTG-resistant kindled rat has also been described (Postma et al., 2000). Animals are kindled by amygdala stimulation in the presence of low concentrations of LTG that do not interfere with the generation of an afterdischarge. Kindled rats are then unresponsive to a later challenge dose of LTG, which would otherwise be effective in blocking fully kindled seizures. This model shows resistance to carbamazepine (CBZ) but not VPA (Srivastava & White, 2013). These observations suggest that seizures induced in this model may be particularly insensitive to ASDs with sodium channel blocking activity. The ASP now frequently uses the LTG-resistant kindled rat as part of routine screening of compounds in an effort to differentiate the pharmacology of the most promising compounds. The clinical relevance of this model to human epilepsy is not known; nonetheless, it provides yet another chronic model wherein the anticonvulsant profile of investigational ASDs can be characterized and differentiated from established ASDs.

**Chemoconvulsant and electrically induced status epilepticus**

The capacity to undertake long-term video-electroencephalography (EEG) recordings from experimental animals is now commonplace. A number of studies have accordingly evaluated the effect of ASDs on the spontaneous recurrent seizures that arise following an initial status epilepticus (SE) induced by either chemoconvulsant (i.e., kainic acid or pilocarpine) or electrical stimulation of the perforant path or other limbic regions. These are often lengthy and expensive studies to perform, not least because the frequency of spontaneous seizures in rodents can be variable (Williams et al., 2009). As of this writing, a comprehensive evaluation of currently available ASDs has not yet occurred in these models. Of interest, however, the Löschner group has shown that rats exhibiting spontaneous recurrent seizures, subsequent to SE induced by either pilocarpine or electrical stimulation, fall into distinct response groups (responders, nonresponders, and partial responders) when treated with either PHT or phenobarbital (PB) (Bethmann et al., 2007). These groups mirror drug-response phenotypes in human epilepsy and, together with PHT- and LTG-resistant kindled rats, may be a useful means of identifying ASDs with preferential efficacy against drug-resistant seizures.

Another chemoconvulsant model with potential utility in the preclinical identification of ASDs with efficacy against drug-resistant seizures is the intrahippocampal kainate model (Riban et al., 2002). Within a few weeks of injection, both rodents show focal epileptiform activity (measured by EEG) arising from the hippocampus and occasional generalized seizures. Although the focal discharges are not associated with behavioral correlates, EEG can be used as a surrogate in efficacy evaluations, and the focal discharges occur with sufficient frequency and duration that medium throughput drug studies can be performed with adequate statistical power (Maroso et al., 2011). This model was instrumental in identifying the preclinical efficacy of the interleukin-converting enzyme inhibitor VX-765, which is currently in clinical trials for the treatment of refractory epilepsy (Ravizza et al., 2006). This model is currently used by a contract research organization, Synapcell, to evaluate traditional ASDs as well as investigational compounds. The electrographic seizures that occur in this model have been found to be responsive to pregabalin (PGB), LEV (at high doses), VGB, diazepam (DZP), and tiagabine (TGB), whereas VPA, LTG, PHT and CBZ are inactive. To date, however, these findings have been published only in abstract form (Langlois et al., 2012).

**Traumatic brain injury**

Traumatic brain injury (TBI) in rodents results in the development of spontaneous focal seizures with very occasional secondary generalization (D’Ambrosio et al., 2009; Kharatishvili & Pitkanen, 2010; Bolkvadze & Pitkanen, 2012). The unpredictable seizure frequency has meant that experiments to characterize the effect of ASDs on spontaneous seizures have been difficult to perform. Nevertheless, some data are beginning to emerge that suggest that established ASDs, including CBZ and VPA, are not able to block the focal neocortical seizures that arise as a result of TBI (Eastman et al., 2010, 2011). Although focal seizures in these models may be drug-resistant, from a practical standpoint the low frequency of tonic–clonic convulsions and technical demands of these models mean that they are unlikely to be used as routine screens for new ASDs, and that they might find their use in the follow-up differentiation process. The search is on for biomarkers, such as altered seizure threshold, which might be employed as indicators of efficacy of potentially novel therapies (Bolkvadze & Pitkanen, 2012).

**In vitro models of drug-resistant seizure-like activity**

Several in vitro models have been evaluated for the drug responsiveness of electrographic measures that are correlated with seizure-like activity (Table 2). The ASP has implemented an in vitro model using brain slices prepared from adult kainate-treated rats in which lowered Mg²⁺ (0.1 mM) and elevated K⁺ (5 mM) concentrations result in recurrent discharges in the entorhinal cortex. These discharges have been shown to be resistant to
therapeutic concentrations of PHT and CBZ, but can be entirely suppressed by ezogabine (EZG) (Smith et al., 2007). Similar observations have been made by Heinemann’s group (Zhang et al., 1995) in slices prepared from naive rats, although the bursts have a shorter latency to onset in slices from kainate-treated animals, increasing the throughput of the assay. Brain slice preparations from human patients following surgical resection of the seizure focus have also been used and activity seems to be resistant to CBZ in these slices, particularly from patients who were clinically unresponsive to the drug (Remy et al., 2003; Jandova et al., 2006). However, given the variable nature of human specimens and unpredictability of surgeries, further development in this approach would be required before this becomes a practical drug development assay. Finally, recent work has focused on electrographic, seizure-like activity in organotypic hippocampal slice cultures as a model of drug resistance as well as epileptogenesis (Dyhrfjeld-Johnsen et al., 2010; Wahab et al., 2010b). Burst firing in the slice culture model is insensitive to a number of conventional ASDs and may thus be a useful medium-to-high throughput screen for identification of novel compounds. As was the case for in vivo models, it is unclear which, if any, of these in vitro models will ultimately prove to be predictive of efficacy against drug-resistant seizures.

**ANIMAL MODELS OF DRUG-RESISTANT PEDIATRIC EPILEPSY**

During brain maturation there is a succession of developmental milestones that are supported by discrete neuronal processes, during several critical periods in life, starting before birth and lasting until adulthood. Disruptions may lead to epilepsy, which is often drug-resistant; in some cases these are due to specific genetic disorders, whereas in others they may be the result of insults suffered during a critical period, with different underlying consequences and thereby different mechanisms contributing to drug responsiveness. During infancy, childhood, and periadolescence, specific epileptic syndromes may occur associated with one or more neurodevelopmental processes, and for each age-group there may be specific comorbidities, consequences, and treatments (Coppola & Moshe, 2009, 2012; Nehlig, 2012). Accordingly, it would be desirable to develop and study models that mimic these age periods, and which take account of gender, when developing novel ASDs for drug-resistant pediatric epilepsy.

Transgenic mouse and model organism technology, combined with advances in our understanding of the complexities of epilepsy genetics, have resulted in a wide array of exciting models of pediatric epilepsies. Mouse models (and in some cases zebra-fish models) for severe myoclonic epilepsy of infancy, benign familial neonatal convulsions, infantile spasms, and tuberous sclerosis have all been generated (Yu et al., 2006; Singh et al., 2008; Price et al., 2009; Chege et al., 2012; Oakley et al., 2013). Many of these genetic models exhibit spontaneous recurrent seizures and provide the research community with an opportunity to identify rational therapies for specific pediatric epileptic syndromes. However, few of these models have been characterized in terms of their responsiveness to existing ASDs (Yu et al., 2006; Oakley et al., 2013; Zhang et al., 2013). As a result, it is currently unknown if these genetic models recapitulate the pharmacoresistant seizure phenotype observed in the corresponding human disorder. In addition, because none of these models are routinely employed in drug discovery, it remains to be seen if they have validity for therapy development.

With respect to pediatric models of induced seizures, there are several that could be considered for use in drug discovery. There are two main concerns with many of these models; ASDs are typically administered before the epileptogenic insult and the seizures are induced on a substrate of a normal nervous system. Such models include febrile seizures induced by heating the animal, injection

### Table 2. Pharmacologic properties of in vitro models of drug resistance

<table>
<thead>
<tr>
<th>Pharmacosensitivity</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human resected brain slice</td>
<td>Resistant to ASD</td>
<td>Slices prepared from resected tissue of CBZ-resistant patients</td>
</tr>
<tr>
<td>Brain slice from KA treated rat – low Mg²⁺</td>
<td>EZG</td>
<td>PHT, TPM, CBZ</td>
</tr>
<tr>
<td>Low Mg²⁺ brain slice</td>
<td>EZG</td>
<td>PHT, TPM, VPA, PB, PHT, CBZ, VPA, PB, DZP, CZP</td>
</tr>
</tbody>
</table>

ASD, antiseizure drug; KA, kainic acid; EZG, ezogabine; CBZ, carbamazepine; VPA, valproic acid; TPM, topiramate; PHT, phenytoin; PB, phenobarbital; DZP, diazepam; CZP, clonazepam.
of kainate or N-methyl-d-aspartate (NMDA), exposure to flurothyl, kindling, and exposure to hypoxic–ischemic conditions (Moshé & Ludvig, 1988; Noam et al., 2013; Galanopoulou, 2013). For the most part, the resulting seizures occur only in response to the insult and do not generally persist or recur spontaneously. Nevertheless, these models reveal discrete windows of responsiveness to various agents, as exemplified by the flurothyl-induced seizure studies performed by Velisek et al. (1995a,b). Hasson et al. (2008) have also shown that the ability to control prolonged kainate- or pilocarpine-induced SE is age dependent, with total failure of the tested drugs in postnatal (P) day 9 rats, whereas the same treatments were effective in P15 and P21 animals. These models represent a viable screen for the acute effects of putative ASDs as a function of age. However, seizures in these models are neither spontaneous nor recurrent and as a consequence do not mimic the phenotype of children with catastrophic epilepsies.

Two models of pediatric epilepsy that result in spontaneous seizures are the tetrodotoxin (TTX) model and a multiple hit model of infantile spasms (Lee et al., 2008; Galanopoulou, 2013). Continuous infusion of TTX into the cortex of young rats results in spontaneous seizures. However, the pharmacologic profile of this model has not been explored. In the multiple hit model of infantile spasms, young rats (typically P3) are injected intracerebroventricularly (i.c.v.) with doxorubicin and lipopolysaccharide, and then 2 days later with the serotonin depletion agent p-chlorophenylalanine (Scantlebury et al., 2010) (Galanopoulou, 2013). These animals subsequently develop spontaneous spasms and cognitive disabilities. The spasms meet the criteria for pharmacoresistance, as they are insensitive to both PHT and adrenocorticotropic hormone (ACTH), a commonly used therapy in infantile spasms (Scantlebury et al., 2010). If the pharmacologic characterization of these genetic and acquired models of catastrophic pediatric epilepsies proves to be consistent with the human phenotype, they should be incorporated into future drug screening approaches in an effort to identify therapies in these rare but nonetheless devastating conditions.

**Available Infrastructure for Drug Development**

The ASP was established in the mid-1970s as a contract between the NINDS and the University of Utah and is the last remaining branch of the Anticonvulsant Drug Development (ADD) program. The ASP has been successful in achieving its primary objective, which was to provide an incentive for drug development for the symptomatic treatment of seizures. The ASP directly contributed to the identification and characterization of nine compounds that are now licensed for the treatment of epilepsy and have provided important preclinical validation for several other approved agents as well as those in the pipeline. While generally better tolerated, it is important to note that the newer ASDs still produce adverse events which can contribute to a poor quality of life (Perucca & Gilliam, 2012) and there still remains a substantial population of pharmacoresistant patients. Thus, the need for novel therapies with better efficacy and improved side effect profiles remains. The ASP was recently reviewed by a group that provided a number of recommendations to enhance outcome in the treatment and prevention of epilepsy. The working group report is available online (http://www.ninds.nih.gov/research/asp/asp_working_group_report_022712.htm). Discussion points are raised in several letters in response to the working group recommendations (Bialer, 2012; French, 2012; Ranganathan, 2012; Shinnar & Glauser, 2012). It is not our intention to revisit the report or the critiques but rather to discuss how the ASP is revising its protocols to increase the likelihood of identifying compounds that will offer significant improvements in treatment.

The issue at hand is defining a pathway that will lead to the identification of more efficacious therapies for the refractory patient. Since the current approach has been successful in its ability to identify mechanistically novel compounds, the currently available models can be used to define efficacy. The problem is that we don’t, at this point, know which molecular mechanism(s), if appropriately engaged, will lead to improved seizure control. As such, there is no real guarantee that any of the models discussed above will be more or less likely to identify a highly effective therapy. In other words, is it the model or the mechanism that continues to elude us? In the absence of more insight into the mechanistic basis of pharmacoresistance, the community needs to be circumspect in its approach to drug discovery and not be too eager to dismiss a process that has, for decades, been extremely successful in bringing new therapies to the patient. Instead, we need to continue to look for complementary approaches and novel models that can be used in parallel to more fully differentiate the antiseizure profile of novel investigational drugs so that the most promising leads are advanced for further preclinical and clinical evaluation.

**Screening strategies for future success at the ASP**

As part of the evolution of the ASP, approaches are being considered that will implement more stringent screening assays into its protocol. The idea is to build on the strengths of the MES, 6 Hz and other protocols and add filters that will quickly advance the most promising, paradigm-shifting investigational drugs. Early screens in the discovery process must be robust enough to capture positive hits, limit false negatives, be efficient in terms of cost and compound availability, and be of medium-to-high throughput. There are currently no validated animal
models of pharmacoresistant epilepsy that meet all of these criteria but those that are well-characterized and considered by the research community to offer predictive value for clinical efficacy are being incorporated and optimized.

Figure 1 illustrates the strategy that has been proposed for implementation of novel screening assays within the ASP. In addition to the MES test, the 6 Hz test becomes part of the initial screening process. The corneal kindled mouse model will also be included at an early stage. Efficacy data in this model correlates exceptionally well to that obtained in the hippocampal kindling model (Rowley & White, 2010) and requires very little compound. Compounds that are effective in any of these initial screens will move on to a phase in which activity is differentiated across a range of models. Compounds will be evaluated in the LTG-resistant kindled rat and the in vitro slice model derived from kainate-treated rats. In addition, compounds will be evaluated for their ability to alter seizure threshold in the intravenous pentylentetrazole (PTZ) test. This test identifies compounds that may lower seizure threshold. A negative outcome in this test would not necessarily halt development; however, it does provide a cautionary signal that should be followed as the compound proceeds into IND-enabling toxicology studies and human development.

Upon meeting prescribed efficacy criteria, compounds will be further investigated in spontaneous seizures models, a pediatric seizure model, and in benzodiazepine-resistant SE. They will also be assessed for their in vitro neuroprotective effects and propensity for cognitive and neuropsychiatric adverse events using relevant models. The aim is to identify molecules that are anticonvulsant in the traditional sense, potentially effective against refractory seizures, are more tolerable, and devoid of the propensity to exacerbate comorbidities.

The ASP and NINDS are working closely with sponsors of compounds that might require more specialized testing, such as chronic dosing regimens or use in specific models. This allows sponsors to engage with and utilize the expertise of the ASP if the standard screening protocol is not appropriate. There may also be the flexibility to establish subcontracts with external laboratories. For example, there is little point in importing genetically engineered models of human epilepsies that display spontaneous recurrent seizures into the ASP when the screening can be performed in their lab of origin. While no such subcontracts currently exist, it is being explored as a way to leverage finite resources in a difficult drug development landscape.

**Partnering with Regulators to Enable New Treatments for Drug-Resistant Epilepsy**

The preclinical development of treatments for drug-resistant epilepsy must be conducted with an understanding of the regulatory requirements for marketing authorization. At the same time, regulators will benefit from an appreciation of developments in preclinical science as they implement changes in policy to enable novel medications to be registered for the treatment of drug-resistant epilepsy. An ongoing dialogue between researchers and regulators is essential to ensure that those regulatory pathways exist and are based on sound evidence.

Marketing authorization of new ASDs is based on well-controlled clinical studies demonstrating safety and efficacy in groups of patients with drug-resistant epilepsy. However, those trials are not powered or designed to assess seizure freedom or efficacy in patient subgroups. Guidance on clinical trial designs to assess efficacy in treating drug-resistant epilepsy does not exist, and we
believe such guidance from regulatory agencies should address the following: (1) preclinical data (if any) to support regulatory decisions; (2) patient selection; (3) clinical end points; (4) study designs and duration. Some of these issues are considered below.

Preclinical studies

The EMA guidance acknowledges that studies on drug mechanism and characterization of drug action in diverse experimental models are important, but there is no mention of experimental studies aimed at assessing potential agents for utility in the treatment of drug-resistant epilepsy. The FDA guidance does not discuss preclinical studies at all. Preclinical studies could provide useful information on issues such as subpopulations that might benefit from a specific treatment, dosing and duration of treatment, appropriate clinical end points, potential biomarkers, and expected adverse effects.

Patient selection

Guidance from both FDA and EMA allows studies of a heterogeneous group of patients with incomplete seizure control or who are experiencing excessive adverse effects from current medication. Unfortunately, studies of this kind ensure that a simple demonstration of efficacy will not provide information on the utility of a new drug in any specific subgroup of patients. When there is data to suggest selective efficacy against a specific seizure type or syndrome, this should guide the design of the clinical development program in terms of patient selection. This may also allow the sponsor a rapid and cost effective route to market by capitalizing on a designation of “orphan drug status.” Under these circumstances, sponsors are incentivized and patients may benefit from a syndrome-specific compound.

Clinical end points

Clinical end points currently focus on seizure reduction in medication resistant patients using the ASD as adjunctive treatment compared to placebo without emphasis on seizure freedom, monotherapy, or the use of biomarkers. Monotherapy studies in newly diagnosed patients typically target seizure freedom, but are not useful in assessing efficacy in medication resistant epilepsy. Seizure freedom in pharmacoresistant patients is an important end point, but one that requires unrealistically large sample sizes. One monotherapy approach currently endorsed by both the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) is “conversion to monotherapy” where patients with drug-resistant epilepsy are randomized to receive either a high or a low dose of the investigational drug (an established ASD may be used as the low-dose comparator instead) (Arroyo & Perucca, 2003). The end point in this design is retention time following discontinuation of baseline medications. This design is seldom used because of ethical considerations, although a trial design based on historical controls may be applicable in some instances (French et al., 2012). In addition to traditional efficacy outcomes, funding organizations should support the development of biomarkers. The accompanying manuscript by Engel et al. (2013) discusses this issue.

**SUMMARY AND RECOMMENDATIONS**

The London meeting participants agree that there is a pressing need for better ASDs to address refractory seizures in patients who are not eligible for surgery. Quality of life for those patients is impacted dramatically by uncontrolled seizures and they are at a higher risk of sudden unexpected death in epilepsy (SUDEP). What is not clear is the route to success for new therapy development for these patients in the current scientific and economic climate. One direction is to screen compounds that are rationally designed using a variety of in vivo and in vitro models that exhibit pharmacoresistant phenotypes. This is an approach that the ASP and the wider epilepsy research community are implementing. The last several decades have seen an enormous increase in the development of genetic and acquired models that recapitulate epilepsy throughout the lifespan. These models have generated new knowledge regarding the basic mechanisms underlying seizure disorders and, in many cases, have identified potential new therapeutic targets. However, there is often little information regarding the pharmacologic profiles of existing ASDs in these models. This gap in our knowledge holds the field back, as it is not clear which models will be most useful in identifying novel therapies. Therefore, we recommend that funding be made available for the comparative characterization studies necessary to assess the suitability of these models for drug discovery. Sadly, grant review panels often regard such studies as lacking innovation and manuscript reviewers can be reluctant to accept negative findings for publication, even though negative data with existing ASDs will be the benchmark for models of pharmacoresistant epilepsy. It is essential that these characterization studies are communicated to the field and the use of searchable, open-access, online databases might be one way to address this concern.

A major concern is the issue of bringing new drugs to a saturated epilepsy therapy marketplace, with many pharmaceutical companies having suspended development of ASDs due to the perceived difficulties in recouping the investment required to obtain a new drug approval. However, there was a sense that commercial enthusiasm might be restored under specific circumstances, as follows: (1) it is likely that any compound with disease-modifying properties would be sufficiently attractive to merit the necessary investment; (2) if an ASD were a novel, first-in-class compound with better tolerability and safety profiles than...
existing agents, there would potentially be support for development. To that end, the Office of Translational Research at NINDS has several programs now in place that can support the early development of innovative new therapies and that limit the economic risks that companies face (http://www.ninds.nih.gov/funding/areas/ translational_research/index.htm); (3) any new treatment that possessed a sufficiently broad antiseizure profile and also mitigated some of the common comorbidities associated with epilepsy would be of interest; and (4) identification of niche compounds with efficacy against a specific seizure type or syndrome in limited patient populations could take advantage of “orphan drug” status, giving sponsors a privileged route to market (given recent dramatic developments in the genetics of epilepsy, it is not difficult to envision the reality and benefits of this approach); and (5) modifications to regulatory statutes and guidelines to incentivize companies to pursue the development of new ASDs (such changes could include increasing patent life, pathways to earlier monotherapy licenses for first-in-class compounds, and approval of a catch-all “epilepsy” indication without specifying age, seizure type, or adjunctive use restrictions).

In conclusion, the participants of the London meeting were enthusiastic about recent advances in basic research that have increased our understanding of the mechanisms underlying epilepsy. These have revealed a number of new directions for preclinical drug development, and although there is much work still to be done, there is also hope that novel, more effective and targeted therapies can be rapidly identified using animal models that are more reflective of the clinical condition. Such compounds have the potential to offer unprecedented opportunities for seizure freedom, improved quality of life, and a reduced risk of SUDEP for patients currently living with refractory epilepsy.

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REFERENCES


