Approaches to seizure risk assessment in preclinical drug discovery

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Assessment of seizure risk traditionally occurs late in the drug discovery process using low-throughput, resource intensive *in vivo* assays. Such approaches do not allow sufficient time to mitigate risk by influencing chemical design. Early identification using cheaper, higher throughput assays with lower animal and compound requirements would be preferable. Here we review the current techniques available to assess this issue and describe how they may be combined in a rational step-wise cascade allowing more effective assessment of seizure risk.

Introduction

Drug-induced seizures are a serious, potentially life-threatening adverse drug reaction (ADR) that can result in the failure of drugs to be licensed for use, or withdrawn from the market. Compounds associated with this liability span a wide variety of pharmacological classes and therapy areas, including many not targeted at the central nervous system (CNS). A set of 266 medicinally relevant compounds that listed seizure or convulsion as an ADR (Appendix A) was assembled on the basis of information available in the Physicians Desk Reference [1], the Infotext section of Micromedex [2] and the BioPrint^{TM} database [3]. Not surprisingly, the largest therapy area represented in this set of seizure-associated agents is CNS disorders (35%). Agents associated with other therapy areas are also well represented, with infection (16%), cardiovascular (14%), respiratory (12%), metabolism (6%), gastrointestinal (4%), cancer (3%), viral (3%) and other (8%) all showing significant numbers of compounds with seizure liability. In agreement with this pharmacological diversity, retrospective analysis of AstraZeneca discovery compounds (1999–2008) revealed that approximately 50% of those affected by seizure in preclinical studies were non-CNS targeted.

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Compounds may impact CNS function directly or via indirect actions, such as metabolic disturbances or effects on the blood–brain barrier (BBB) [4–6]. The term seizure refers to a period of rhythmic, synchronized abnormal neuronal activity that, depending upon the brain region concerned, may result in a number of symptoms including visual disturbances, tingling, mood changes or the more obvious events typical of a classic tonic–clonic convulsion. This latter event is characterized by muscle rigidity followed by large amplitude rhythmic jerking movements. A convulsant compound is one that induces overt motor effects of this type; a proconvulsant compound increases the likelihood or severity of a convulsion. A proconvulsant drug may seem to have no effect when seizure-precipitating factors are absent; the effect may only be seen when other conditions have altered the normal balance of inhibition and excitation in the brain (e.g. epilepsy, stress, presence of other proconvulsant compounds). Convulsant compounds are typically proconvulsant at lower doses than the dose that produces convulsion. It is important to note that seizure activity is not always followed by the behavioral changes that define a convulsion.

Owing to the potentially serious consequences of drug-induced seizures, significant effort is expended in preclinical safety assessment to identify and mitigate this effect. Despite the importance of this issue, however, there are no regulatory guidelines

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describing how drug-induced seizure should be addressed. Before the year 2000, seizure risk was typically addressed preclinically as part of a larger systematic investigation of CNS risk [7]. With the release of ICH S7A guidelines, the comprehensive testing changed to a streamlined package geared to meet the minimal requirements of regulatory agencies [8]. But these lean packages fail to detect seizure liability until late discovery phases, when it is typically revealed in repeat-dose toxicity studies. This motivated us to develop a strategic approach to identify seizure liability in early discovery for projects or chemistry of highest risk. Early hazard identification allows time for a project to mitigate risk through improved chemistry or pharmacokinetic approaches. In this review we discuss the tools available to address this risk and describe how they may be combined in a rational step-wise screening cascade for effective assessment of seizure liability before first time in man (FTIM).

In silico approaches

Computational models

Computational models have been utilized successfully in preclinical safety assessment where a strong linkage between a particular molecular target and a clinical adverse event has been demonstrated. A good example of this is the interaction with the hERG-encoded potassium channel; compounds that block this channel are associated with *torsade de pointes* [9,10]. Here, predictive computational models have been developed that allow compounds to be virtually screened as part of the design process, increasing the likelihood that interaction with the hERG channel will be avoided [11].

We have identified a number of pharmacological targets that may play a role in drug-induced seizure (Table 1; a detailed description of how these targets were identified can be found in the legend), however, predictive computational models, such as those developed for hERG, are not as readily available for most of these. The current lack of evidence with respect to which of these molecular targets, or sets of targets, are of greatest physiological relevance in eliciting a seizure response adds a further complication. Computational prediction of seizure liability therefore awaits both an improved biological understanding of the relevance of these pharmacological targets and developments in our predictive secondary pharmacology abilities. In addition to computational models for specific pharmacological targets, *in silico* models of brain/plasma partitioning may be used as a first tier screen [12]. Compounds predicted to penetrate the BBB and, therefore, have high CNS exposure should be scrutinized more carefully than those with low CNS penetration. However, low CNS penetration does not guarantee low seizure liability since BBB function can be significantly impaired in owing to pathophysiology [13] or drug-induced changes [14,15] in certain patient populations. Such conditions are not considered in BBB penetration models that only assess penetration of the healthy BBB.

Computer simulation of seizure

Seizure is a complex network phenomenon underpinned by the activity of numerous molecular targets resulting in the synchronous firing of neurons. It is possible to use computer modeling to simulate brain function from the individual neuron to neuronal networks and computer models of seizure, in particular reflecting the disease state of epilepsy [16]. Such tools have been used to model the physiological conditions required for epileptic discharge [17], transition from clonic to tonic activity [18] and onset of partial seizure, as in temporal lobe epilepsy [19]. Although *in silico* approaches can be useful in modeling the physiological mechanisms underlying seizure induction, it is difficult to predict how compounds may alter these processes. To enable this connection to be made, the mechanism by which pharmacological modulation of a single or multiple molecular targets is translated into function must be established. While this connection is not well enough understood at present, considerable excitement exists for scientific growth in this direction.

In vitro assays

Pharmacological profiling

The CNS expresses a multitude of drug targets including voltage-gated and ligand-gated ion channels, G-protein-coupled receptors, enzymes and transporters, both on neuronal and non-neuronal tissues. Pharmacological modulation of any of these targets may have effects on brain function, including seizure induction. Pharmacological profiling of compounds, using high-throughput radioligand binding assays, enables binding potency at these targets to be assessed. Owing to low cost, low compound requirements and rapid turnaround time, this can be conducted at the early phases of drug discovery [20]. Discovery compounds are typically profiled at >100 targets covering a broad spectrum of pharmacological diversity. In order to assess which of these targets may be most strongly associated with seizure risk we performed a detailed literature review and assembled a list of 53 targets, which were classified as being associated with drug-induced seizure. A summary of the review described is presented in Table 1. Clearly, the strength of this approach is limited by the available scientific literature and the search tools at our disposal. It is possible that some targets with a seizure risk potential were not identified owing to a lack of scientific knowledge of these targets or availability of selective tool compounds.

Identification of off-target binding at any of these 53 targets may provide the first indication of potential seizure liability *in vivo*. Activity in binding assays can be followed up with functional assays to identify whether the compound has agonist or antagonist activity at the target of interest. As with all preclinical safety data, it is important to consider these data in the context of efficacious exposure values. A low margin between this and activity in pharmacological profiling assays would indicate an increased seizure risk. Armed with this information, a plan to address target-related seizure liability early in the testing cascade can be formulated and implemented in a timeline most likely to benefit the project. Given the complexity of drug-induced seizure and the observation that binding activity at these targets does not guarantee a seizure risk, data should be used as a flag for potential activity and may also be useful in compound prioritization.

In vitro *electrophysiology*

Abnormal seizure-like activity can be induced and recorded from *in vitro* brain slice preparations using electrophysiological techniques. Such preparations have been shown to detect effects of a number of neurotoxins [21,22], including convulsant compounds [23–25]. In agreement, we have described a rat hippocampal slice

TABLE 1

Pharmacological targets with a positive association to drug-induced seizure

	Link to seizure			
	Pharmacological	**Genetic**	**Antiepileptic drug**	**Bioprint™**
G-protein-coupled receptors				
5-HT_{1A-B}	+			+
5-HT_{2A}				+
5-HT_{2C}	+			+
5-HT_6				+
5-HT_7	+			+
Adrenergic $\alpha_{1A,\ 2A-C}$				+
Adrenergic β_{1-3}				+
Cannabinoid CB_1	+			+
Dopamine D_1	+			+
Dopamine $D_{2,\ 3,\ 4.4}$				+
Growth hormone secretagogue				+
Histamine $H_{1,2}$				+
Melanocortin M_1				+
Muscarinic acetylcholine M_1	+			+
Muscarinic acetylcholine M_{2-5}				+
Opioid δ	+			
Opioid κ				+
Sigma Nonselective				+
Urotensin UT_1				+
Ligand-gated ion channels				
5-HT_3				+
AMPA	+		+	
$GABA_A$, agonist site	+	+		
$GABA_A$, Flunitrazepam site	+	+	+	
Glycine, strychnine site	+			
Kainate	+		+	+
Nicotinic acetylcholine	+	+		
NMDA, agonist site	+		+	
NMDA, glycine site	+		+	N/A
NMDA, phencyclidine site	+		+	N/A
Voltage-gated ion channels				
K_{ATP} potassium channel	+	+		+
Sodium Channel Site 2	+	+	+	+
L-type calcium channel diltiazem site				+
L-type calcium channel verapamil site				+
K_A potassium channel	+	+		
KNCQ2/3 potassium channel	+	+	+	N/A
Transporters				
5-HT transporter	+			+
Noradrenaline transporter	+			+
Dopamine Transporter				+
Enzymes				
Acetylcholinesterase	+			

5-HT: 5-hydroxytryptamine. AMPA: α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate. GABA: γ-aminobutyric acid. NMDA: *N*-methyl-D-aspartate. N/A: Data not included in Bioprint™ database.

Secondary pharmacological targets with a positive link to drug-induced seizure. Compounds were classified according to 4 criteria:

- **Pharmacological**—is there published evidence that pharmacological modulation of the target induces seizures *in vivo*? Evidence usually involves the use of tool compounds with known selectivity for particular targets but may also be obtained from transgenic animals.
- **Genetic**—are mutations in the gene encoding the molecular target associated with epilepsy in man?
- **Antiepileptic**—is the target the site of action of anti-epileptic drugs? Anti-epileptic drugs cause therapeutic effects by pharmacological modulation of a number of molecular targets. It is reasonable to suspect that reverse modulation of the same target may induce seizure.
- Bioprint™—is there a statistically significant association between the target and clinical reports of seizure/convulsion? To assess this we used the Bioprint™ database that contains the *in vitro* binding profiles of more than 2300 marketed compounds across a range of approximately 200 targets together with clinical safety information including the frequency of convulsions. The association between the search term 'convulsion' and each target was assessed using 'assay by Adverse Drug Reaction (ADR)' function of Bioprint™. In a separate analysis, we used the Bioprint™ database to obtain the *in vitro* binding profiles of 266 compounds in our seizure set (see Introduction). Compounds were considered to be active in a given assay if their percent inhibition was 50% or greater. These data were then analyzed to determine which binding activities are statistically more highly represented in the seizure-associated compounds.

A positive in at least one criterion was taken as evidence of a possible link to drug-induced seizure (positives are labeled '+', the lack of symbol indicates no link to that criterion). This analysis does not provide irrefutable evidence of an association to seizure; the strength of the link clearly increases as the number of criteria matched increases (note the high prevalence of ligand-gated and voltage-gated ion channels). A more definitive answer may be reached by testing selective tool compounds for these targets in seizure models or, where pharmacological tools are limited, by examining the phenotypes of genetically modified mice.

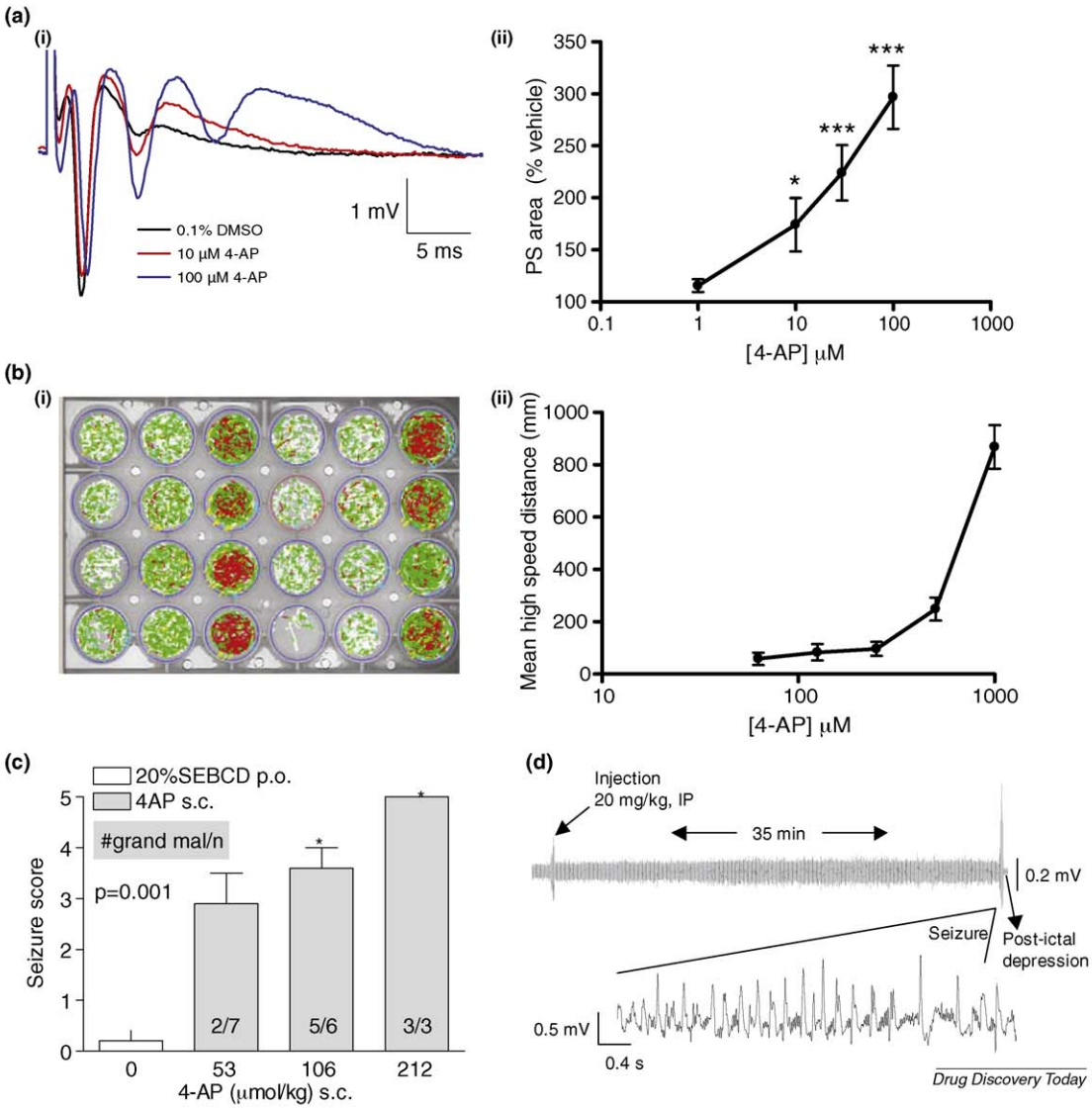

FIGURE 1

Effects of 4-aminopyridine (4-AP) in four seizure liability assays. (A) *In vitro* rat hippocampal slice [26]. Hippocampal brain slices were prepared from Han Wistar rats and CA1 population spikes (PS) were evoked every 30 s by electrical stimulation of the Schaffer collateral pathway using bipolar tungsten electrodes. (i) Representative PS recordings in the presence of vehicle (0.1% DMSO), 10 μM and 100 μM 4-AP, note the appearance of additional population spikes in the presence of 4-AP. (ii) Mean concentration–response data ($n = 11$ slices, from three animals). (B) *In vivo* zebrafish larvae [36]. Briefly, larval locomotor activity was analyzed (1 animal per well) in 24 well plates using automated videotracking software. (i) A typical 24 well plate system output: each column represents 4 replicate animals exposed to one of five test concentrations (the highest test concentrations in columns 3 and 6), together with a control group (column 4). White–Green–Red tracking lines signify movements of increasing speed. (ii) Mean concentration–response data ($n = 12$ larvae per concentration). (C) Mouse spontaneous seizure. CF1 male mice were subcutaneously dosed and observed for seizure-related behaviors and scored for severity (0 = normal, 1 = facial clonus, 2 = myoclonic jerk, 2.5 tonic forelimb extension, 3 = forelimb clonus, 4 = rearing/loss of righting, clonus 5 = tonic hindlimb extension). '*grand mal*' was defined as score > 2.5. (D) Mouse EEG. Example of a 4-AP induced seizure from C57BL6 mouse implanted with cortical electrodes. Spike-wave activity was contemporaneous with a loss of righting response, followed by forelimb clonus and ending with tonic–clonic convulsion.

assay that can detect the effects of a wide range of compounds associated with seizure induction in man [26]. Specifically, pro-convulsant compounds were shown to potentiate electrically evoked CA1 population spikes (Figure 1A). To date, most work in this area has focused on the hippocampal brain slice, largely owing to its defined cytoarchitecture that makes it amenable to electrophysiological recording [27]. The hippocampus is also strongly linked to partial seizures, including temporal lobe epilepsy [28]. Compounds may, however, elicit seizure via a number of different brain regions; for example, generalized seizures are thought to result from changes in the electrical activity of thalamocortical circuits that lead to simultaneous disruption of normal brain activity in large areas of the brain [29]. For this reason, there may be utility in further investigating the use of brain slices obtained from other brain regions. The most appropriate preparation is likely to be dependent on the exact mechanism of drug-induced seizure. In addition to standard single-electrode brain slice electrophysiology, multi-electrode arrays can be used to

measure electrical activity across a whole brain slice allowing the epileptic focus to be identified and the spread of excitation to be visualized [30]. Furthermore, a recent development with potential value in screening for seizure liability involves the use of re-aggregated brain cell cultures placed on a multi-electrode array [31].

In vivo methods

Zebrafish

In recent years, the zebrafish (Zf.; *Danio rerio*) has emerged as a potentially valuable animal model for frontloading human drug safety assessment (see [32,33] for reviews). Of the endpoints reported, seizure liability represents one of the most promising areas of interest. Exposure of zebrafish larvae (7-day-post-fertilization) to pentylenetetrazole (PTZ) induces abnormal behavioral activity in a concentration-dependent manner. Specifically, PTZ exposure resulted in dramatically increased swimming speed/activity in combination with rapid circling, followed by a loss of posture at higher concentrations [34], suggestive of a clonus-like convulsion. Furthermore, electrophysiological recordings in adult Zf revealed electrographic discharges consistent with ictal events. Subsequently, it was reported that this convulsant activity could be blocked by exposure to antiepileptic drugs (AEDs) [35]. In terms of preclinical drug discovery, these findings are of particular interest as locomotor activity can be easily quantified using automated videotracking and image analysis (Figure 1B). Consequently, we have developed a medium-throughput *in vivo* Zf screen for the identification of convulsant liability on the basis of the quantification of high-speed locomotor activity in 7-day-post-fertilization Zf embryo–larvae [36].

Early in vivo *studies*

Efficacy and drug pharmacokinetic and metabolism (DMPK) studies are typically carried out early in the drug discovery process before standard safety studies. Behavioral observations in these studies may provide the first evidence of potential seizure liability. Therefore, it is important to train scientists involved with these studies to recognize behaviors that may result from seizure activity, particularly when this is not the primary aim of the study.

Standard safety studies

Early assessment of *in vivo* neurotoxicity can be achieved using multiparameter behavioral tests, such as a modified Irwin assay [37] or a functional observational battery (FOB) [38]. These studies are a mandatory part of the regulatory safety pharmacology package (ICH S7A [8]). These models examine a variety of behavioral endpoints to detect changes in locomotor function, muscle strength, co-ordination, and autonomic control. In comparison to specific seizure studies, multiparameter screening in rodents has a low sensitivity for detection of convulsant liability (unpublished observations from numerous AstraZeneca preclinical projects). There are various possible reasons for this. Firstly, these assays use few fixed time points thus precluding detection of convulsions during an unobserved period. Secondly, seizures may be difficult to distinguish from more general behaviors. Thirdly, such studies only involve a single administration of a test compound, whereas a proconvulsant compound may require repeated dosing to increase the likelihood of detecting a seizure. It is well known that a non-convulsant acute dose of a compound may induce convulsion when administered chronically, the effect is commonly referred to as chemical kindling [39]. Finally, it may be necessary to disturb normal brain homeostasis in order to reveal proconvulsant liability. In addition to multiparameter behavioral studies, safety pharmacology studies may be refined to better identify seizure following acute administration, for example using a mouse spontaneous seizure assay (Figure 1C) that is designed specifically to detect this effect.

In addition to standard safety pharmacology studies, regulatory guidelines also require preclinical toxicological testing following acute and chronic dosing in rodent and non-rodent species, usually rat and dog (ICH M3 [40]). Again, these studies may provide useful information regarding potential convulsant liability. In particular, dogs have been shown to be particularly sensitive to drug-induced seizure (unpublished observations from numerous AstraZeneca preclinical projects). This may, partly, be due to a relatively high background level of seizures in inbred preclinical species, as high as 6% in the beagle dog [41–43]. For this reason, animals should be pre-screened to allow those with high background levels of spontaneous seizure to be excluded. Despite these drawbacks, standard toxicology studies may still provide useful information particularly if observations are made at key pharmacokinetic time points such as T_{max}.

Rodent precipitant models

Owing to the lack of sensitivity, standard safety studies are not sufficient to rule out convulsant risk and additional seizure-focused models are required. Quantitative precipitant challenge assays classically applied in antiepileptic efficacy testing have been used to assess proconvulsant risk with some success [44–46]. The most commonly used precipitants are chemoconvulsants and electrical stimulation [47], although other precipitants have been identified (e.g. sound, shaking) [48,49]. In each method, the sensitivity of the animal to precipitant-evoked seizures is established. The test compound is then dosed followed by challenge with the precipitant. A convulsant compound would be associated with a lowering of the threshold to the precipitant challenge. Of these precipitant models, the chemoconvulsant $GABA_A$ antagonist PTZ is the most commonly used. We have found this chemoconvulsant-based assay to be more sensitive than multiparameter screening or using electrical stimulation as the precipitant (unpublished observations). Testing over a range of doses/exposures increases assay sensitivity to proconvulsant risk, but also increases resource demand and animal use. Best results are obtained through continuous behavioral monitoring of animals between dose and subsequent challenge; this can be done relatively easily using video monitoring of the home cage. The latency to abnormal home cage behaviors can then guide the timing of precipitant challenge or *in vivo* electrophysiology recordings.

Electroencephalogram (EEG)

A seizure is defined as abnormal synchronous neuronal activity and, as such, it can be detected by measuring the electrical activity of the brain. The electroencephalogram (EEG) is the gold standard for seizure detection, preclinically and clinically. An excellent compilation on current human EEG techniques is available [50].

The EEG is particularly useful when evaluating compounds that can trigger abnormal behaviors that are not the result of synchro-

nized seizure activity in the brain [51]. Conversely, some compounds may cause seizure activity in the brain without eliciting overt behavioral manifestations and thus can only be detected via an EEG [52,53].

A seizure recorded with cortical electrodes typically manifests in the EEG trace as repeated spike-waves (Figure 1D). These spikes are thought to represent the summated synchronous firing of pyramidal neurons that span the cerebral cortex of all mammals [54]. Spike-wave bursts are remarkably similar across mammalian species with spike or wave amplitudes ranging from several hundred microvolts up to 2 mV and frequencies oscillating between 2.5 and 7 Hz [52]. Regardless of where or how a seizure is initiated (e.g. chemical insult, physical trauma, epilepsy), once it generalizes, the spike-wave morphology on the EEG is easily recognizable [52]. For all these reasons, EEG detection of seizures is a highly translatable biomarker.

In addition to spike-waves, other abnormal waveforms on the EEG can be suggestive of seizure or inter-ictal (between seizures) activity. Seizure-associated abnormalities in the EEG trace almost always involve increases in amplitude, consistent with the excessive excitation of relatively large neuronal populations. Depending on the type of seizure, various other morphologies can be identified such as the polyspike-wave, sharp wave, fast spike, spike-slow wave, and so on [55]. In addition, following a *grand mal* seizure one can often see a period of significant amplitude decrease, typically lasting two to five minutes, described as post-ictal depression [56]. Although EEG recordings can provide definitive evidence of seizure activity, they are not free from artifact or ambiguous interpretation. Thus, it is recommended that EEG studies be accompanied by video monitoring and whenever possible, such recordings should be digitally synchronized to the EEG recording. Spike-wave EEG along with video of behavioral manifestations such as clonic–tonic convulsions constitutes irrefutable evidence of seizure.

There are a number of animal models of chemically induced seizures against which proprietary compounds can be evaluated using EEG. Robust, bilateral spike-wave seizures can be triggered via different mechanisms with the $GABA_A$ receptor antagonist PTZ, the potassium channel blocker 4-aminopyridine, or the glycine receptor blocker strychnine, in all preclinical species including rat and mouse. Nicotine at relatively high doses can induce generalized seizures in rodents that are clearly manifested on the EEG, but are relatively short in duration and allow full recovery of the animal, thus making it a useful chemical model in preclinical studies [57]. Partial or focal seizures can be induced and monitored on adequately positioned EEG electrodes with intracranial delivery of penicillin [58].

Benchmarking of seizure assays

There is no regulatory guidance concerning the preclinical investigation of seizure liability. In order to establish one possible approach, we benchmarked a number of preclinical assays with

TABLE 2

Benchmarking of four preclinical seizure assays using 16 reference compounds

Compound	Assay			
	Rat brain slice	Zebrafish	Spontaneous seizure (mouse)	EEG (mouse)
4-Aminopyridine	+	+	+	+
Aminophylline	+	+	+	+ [59]
Bupropion	–	–	+	NT
Cefazolin	+	–	–	+ [60]
Chlorpromazine	+	–	+	+ [61]
Enoxacin	+	+	+	NT
Isoniazid	+	–	+	NT
Maprotiline	–	+	+	+
Meperidine	NT	NT	+	NT
Pentylenetetrazole	+	+	+	+
Pergolide	NT	NT	+	NT
Pilocarpine	–	NT	+	+
Reserpine	NT	NT	+	NT
SNC-80	+	NT	+	+ [62]
Strychnine	+	+	+	+ [63]
AZ12193330[a]	+	NT	+	NT
Total compounds tested	19	25	16	7
Predictability (%)	89	72	94	100

+ Compound correctly classified, – compound incorrectly classified. NT compound not tested.

Benchmarking of four preclinical seizure assays using 16 reference compounds. With the exception of AZ12193330, all compounds are known to induce seizure in man. AZ12193330 was included as a negative control, this compound has been shown to be inactive in all preclinical studies to date. Compounds were tested in: *in vitro* rat hippocampal brain slice, *in vivo* zebrafish larvae assay, mouse spontaneous seizure model and mouse electroencephalogram (EEG) (see Figure 2 for representative data). The predictability value for each assay refers to the total dataset for each assay (some data not shown). The ultimate aim is to expand this dataset to include a larger number of negative control compounds (i.e. compounds that do not induce seizure in man) such that the translation of each preclinical model to clinical outcome can be assessed accurately. Data were generated at AstraZeneca except where referenced.

[a] Negative control compound.

standard compounds known to induce seizures in animals and/or in man. Compounds were chosen to include a wide variety of pharmacological classes and were tested in: Zf larvae, rat hippocampal brain slice, mouse spontaneous seizure and mouse EEG models. Assay predictability (i.e. % compounds correctly identified as true positive or negative) was evaluated together with assay throughput and resource requirement to determine the appropriate use of these assays in drug discovery. A summary of this information is shown in Table 2. A number of compounds were shown to be active across all the assays tested (see Figure 1 for an example of this). In terms of predictability the assays were ranked: mouse EEG > mouse spontaneous seizure > rat brain slice > Zf larvae. Note that the compounds tested were primarily positive control compounds, to further refine this process it would be advantageous to identify true negative control compounds and to benchmark these in the same assays.

One approach to the assessment of seizure risk

The proposed strategy is a step-wise cascade of assays placed according to throughput, compound requirement, turnaround time and predictability. In early discovery the focus being on highly sensitive, high throughput assays with an increasing need for high predictability in the latter phases. An example decision tree is outlined in Figure 2 although this is not an exhaustive description of how the screening cascade could be utilized. The exact use of assays is very much dependent on the results obtained as the assays are conducted or on previous experience with particular targets or chemical series. For example, for a back-up project where *in vitro* and *in vivo* assays have been demonstrated to be well correlated it may be appropriate to select compounds based on results from one *in vitro* assay. Alternatively, previous data may indicate that *in vitro* testing is not predictive, in which case, early *in vivo* testing would be more appropriate. It is important to note that in addition to the seizure assays described, core safety studies should be adapted where necessary to include the use of frequent/continuous behavioral monitoring or EEG collection.

Early in drug discovery, the process begins *in cerebro* with the assessment of target-related risk before compound synthesis. Scientific literature, patent searches, and internal legacy data typically form the foundation of this inquiry. Strong evidence of a target-related liability would trigger early screening in higher throughput, low resource intensive assays such as *in vitro* brain slice or zebrafish (Figure 2). In some cases, particularly for new drug targets, there may be insufficient literature to enable the target-related risk to be assessed accurately. In these cases, an early indication of liability may be abnormal behaviors noted in efficacy or DMPK studies *in vivo*. A single observation of convulsion is not necessarily an automatic trigger for seizure-specific studies. Observations using larger animal numbers across species with exposures close to therapeutic levels would, however, trigger seizure-specific testing to reduce risk to humans. These observations would also probably lead to early investigative studies using *in vitro* models.

Early indication of seizure risk may also arise via pharmacological profiling experiments typically carried out routinely as chemical leads are identified. A lack of binding to secondary targets or large margins to predicted therapeutic exposure, may be sufficient to progress through to the standard regulatory package described in ICH S7A and ICH M3 [8,40]. A binding profile indicating

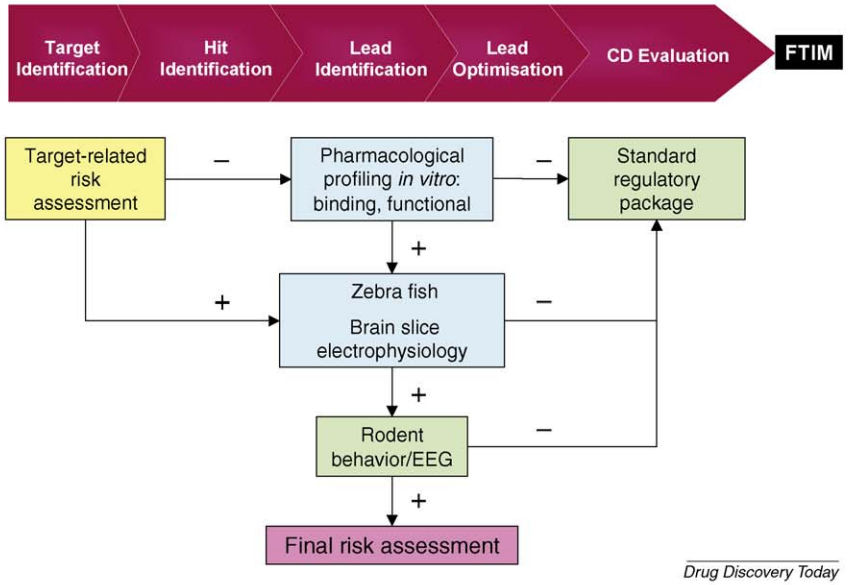

FIGURE 2

Step-wise approach to seizure risk assessment. The proposed strategy is a step-wise cascade of assays prioritized according to throughput, resource requirement and predictability. One example is shown, although the placement of assays is flexible depending on the results obtained or other available information. If the target-related risk is negative, seizure-specific studies would not be performed unless deemed necessary following pharmacological profiling. If a target-related risk is identified, *in vitro* seizure assays would be utilized early and followed up with *in vivo* assays, if necessary. At each stage, negative results would give further confidence to progress to standard regulatory studies. Positive results would need to be considered together with predicted efficacious exposure and therapeutic indication to produce a final risk assessment. Although, the decision to proceed with or stop drug development could be made at any stage, the confidence to do so increases as data are accumulated and a full risk assessment can be made.

affinities to seizure-linked targets (Table 1), particularly within the therapeutic range, would trigger a cascade of studies (Figure 2). In some cases, seizure risk may not be identified until standard regulatory studies; in these cases, more specific seizure assays should be used to further investigate these effects in terms of mechanism and to define safety margins accurately. The final assessment of seizure risk should be an integrated assessment of all the data available, also taking into account DMPK properties (e.g. blood–brain barrier penetration) and predicted CNS exposure for efficacious and seizurogenic effects. If safety margins are deemed sufficient then compounds may still progress to the clinic, although careful monitoring in phase I trials would be essential. Note that there is no regulatory guidance concerning acceptable safety margins to seizure. This is highly dependent on the therapeutic indication, target patient population and competitive environment.

Concluding remarks

Drug-induced seizure is a complex phenomenon that has traditionally been assessed late in discovery using *in vivo* behavioral models. As with other areas of the drug discovery process, there is pressure to identify seizure risk earlier while using fewer resources than with traditional *in vivo* approaches. We have described one possible approach using the assays at our disposal, with a particular emphasis on electrophysiological approaches that are most sensitive but have not been well utilized in this area to date. The value of such an approach will only be realized as data are obtained and the relationship between preclinical and clinical data is assessed, in addition animal usage will be reduced. Ultimately, our aim is to focus on early identification of seizure liability, thereby reducing the risk of observing convulsions in regulatory toxicology studies. Moreover, these efforts will serve to protect healthy volunteers and patients while minimizing the disruption of compound registration due to unexpected convulsions.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.drudis.2009.06.003.

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