Toxicology Research

REVIEW

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Innovative models for *in vitro* detection of seizure

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Data show that toxicity to the central nervous system (CNS) is the most frequent cause of safety failures during the clinical phase of drug development. CNS endpoints such as seizure pose a safety risk to patients and volunteers and can lead to a loss of competitiveness, delays, and increased costs. Current methods rely on detection in the nonclinical rodent and non-rodent studies required to support clinical trials. There are two main issues with this approach; seizure may be missed in the animal studies and, even if seizure is detected, significant resource has already been invested in the project by this stage. Thus, there is a need to develop improved screening methods that can be used earlier in drug discovery to predict seizure. Advances in stem cell biology coupled with an increased understanding of the role of ion channels in seizure offer an opportunity for a new paradigm in screening. Human derived induced pluripotent stem cells (hiPSCs) representative of almost all cellular subtypes present in the brain can be incorporated into physiologically relevant *in vitro* models that can be used to determine seizure risk using high-throughput methods. Akin to the success of screening against a panel of ion channels such as hERG to reduce cardiovascular safety liability, the involvement of ion channels in seizure suggests that a similar approach to early seizure detection is valid. Profiling of the ion channels expressed in hiPSC models showing the seizurogenic phenotype coupled with electrophysiological assessment of ion channel function could translate into an ion channel seizure panel for rapid and reliable *in vitro* detection of seizure. The mechanistic information gathered would support optimal drug design early in development before resources, animals and time have been wasted.

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Introduction

Detection of neurotoxicity induced by prospective new therapies represents a major challenge due to the morphological and physiological complexity of the central nervous system (CNS). Characterised by uncontrolled electrical activity in the brain, seizure liability remains a significant cause of attrition in drug discovery and development. An industry survey published in 2016 reported that seizures were the most commonly encountered CNS issue in preclinical drug development,[1] and between 2005–2010 the CNS was the organ system most frequently associated with safety failure in clinical development.[2] These preclinical and clinical failures lead to a loss of competitiveness, delays and increased costs for the pharmaceutical industry. Compounds associated with this liability span a wide variety of pharmacological classes and therapy areas, including many not intended to target the central nervous system.[3]

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Limitations of current seizure-detection models

CNS toxicity testing already forms part of the "core battery" of safety pharmacology studies specified under the International Council on Harmonisation (ICH) guidelines.[4] However these studies are generally performed by single dose administration and as such adverse effects on the CNS may not be identified until the repeat dose nonclinical rodent and non-rodent studies required to support clinical trials.[5] Unusual movements noted in repeat dose studies indicative of CNS activity would usually trigger a follow-up electroencephalogram (EEG) study to confirm seizure-like activity.[6] Even then, CNS-related signs may be overlooked since they could be sporadic and subtle. Although some progress has been made using in-life detection of seizure using automated video systems that record and analyse animal movements,[7] it would be far preferable to have an earlier prediction of seizurogenic risk that could be used to eliminate liabilities early in discovery while there are still options in chemistry.

Two *ex vivo* approaches are often employed in the CNS screening cascade. These are the rat *ex vivo* hippocampal slice assays and primary rodent cultures, both of which are used to investigate seizurogenic mechanisms. However, both raise con-

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cerns regarding their relevance to humans, use of animals, efficiency and cost.[5] The use of a larval zebrafish locomotor assay has also been explored for assessing seizure liability, but this is a medium-throughput assay and misclassifications occur.[8] Overall, there is a clear and unmet need for earlier and more predictive seizure detection with reduced reliance on costly animal studies with questionable translation. Naturally, this has led to an interest in the development of human *in vitro* models that more accurately recapitulate human physiology and have potential for a high-throughput approach amenable to compound screening.

Potential to develop more relevant human-based models

Recent advances in stem cell and cell culture biology have opened exciting new research areas in many disciplines. Human derived induced pluripotent stem cells (hiPSCs)[9] have revolutionised research and are now increasingly used for disease modelling and toxicology screening. Likewise, these models have considerable promise for neurotoxicity testing including seizure detection.[10] Significant advancements in differentiation protocols mean that it is now possible to create almost all cellular subtypes present in the brain[11–14] providing the opportunity to address the challenge of developing physiologically relevant *in vitro* models. This approach has been adopted by the NC3Rs CRACK IT challenge which focusses on the generation of human 3D stem cell-based models to identify neurotoxicity (including seizure liability) *in vitro*.[15] This challenge is currently underway and has utilised microfluidic OrganoPlate technology to sustain the growth of miniature 3D tissue models comprising multiple iPSC-derived cell types to mimic brain physiology. Compound effects were investigated using real-time and endpoint assays, including calcium imaging, cell viability and neurite outgrowth. Addition of known neurotoxicants (methylmercury, endosulfan, and 2,5-hexanedione) decreased cell viability and neurite outgrowth (methylmercury only) in a concentration dependent manner. Calcium imaging of the miniaturised 3D models revealed that addition of GABA (100 μM) and tetrodotoxin (1 μM) inhibited neuronal firing, thereby demonstrating the potential of this system to detect neurotoxicity and seizurogenic liability.[16] The advantages and disadvantages of currently used nonclinical seizure models and hiPSC-neuronal cultures are presented in Table 1.

Table 1 Advantages and disadvantages of nonclinical seizure models

Nonclinical seizure model	Advantages	Disadvantages
Primary rodent neuronal cultures	-Mature cells -Defined culture methodology -Well characterised	-Difficult to culture -Short lifespan in culture -Low throughput -Isolated cells -Questionable translation to humans -Requires animal sacrifice
Rodent *ex vivo* hippocampal slice assay	-Quick turnaround time -Cytoarchitecture and synaptic circuits intact	-Low throughput -Questionable translation to humans -Requires animal sacrifice
Larval zebrafish locomotor assay	-Medium throughput -Readily available and quick gestation -Inexpensive	-Questionable translation to humans -Misclassifications possible -Not mammalian
Repeat dose nonclinical studies	-In life study -Complete physiological system	-Low throughput -Expensive -Questionable translation to humans -Misinterpretation possible -Requires animal sacrifice
hiPSC-neuronal cultures	-High throughput possible -Defined culture methodology -No barrier to supply -Can create co-cultures and 3D models -Human based model -Can cover healthy donors and patients with specific disease backgrounds -3Rs benefits	-Not fully characterised -May possess embryonic phenotype -Isolated cells -Currently expensive

A mechanistic approach to nonclinical seizure detection

In the search for potential new therapies, a screening tool that also provides mechanistic information on why the compound is seizurogenic would be invaluable. An ion channel focussed approach may be the way forward as there is clear evidence for the involvement of ion channels in seizure. Genetic studies have pointed towards a role for voltage-gated sodium and potassium channels, as well as the ligand-gated ion channels, such as GABA-A and nicotinic acetylcholine receptors.[17,18] Pharmacologically, several ion channel modulators are known to be seizurogenic such as chlorpromazine. Although the development of iPSC-derived neuronal cell models has already shown potential to dramatically improve *in vitro* detection of seizure, there is still a long way to go in terms of characterisation of the ion channel expression profile in these cells. This is no small task considering that it is highly likely that the different differentiation protocols used will produce cells with varying expression profiles which may evoke different cellular responses to test compounds. This exemplifies the importance of characterising the ion channel expression profile of the cells used in screening studies so investigators are fully informed of

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which disturbances to ion channel functionality will or will not be detected using the iPSC-derived models. Moving forward, ion channels that are linked to seizure could be transfected into a routinely used cell line in order to create a full ion channel panel for *in vitro* detection of seizure. The approach based on determining the seizurogenic risk of ion channels expressed in hiPSC-neuronal cultures through to creation of an *in vitro* ion channel panel for early detection of seizure is summarised in Fig. 1. Such a model would allow assessment early in the drug discovery process of whether compounds are likely to cause seizure, allowing for chemical modification in the make-test cycle. Additionally, an ion channel panel that predicts seizure has utility in the development of novel anti-epileptic medicines.

Benefits of an integrated electrophysiological-ion channel *in vitro* screening approach

In a similar manner to the success of screening against a panel of ion channels to reduce cardiovascular safety liability,[19] and the more recent comprehensive *in vitro* pro-arrhythmia assay (CiPA) initiative using hiPSC-cardiomyocytes,[20] increasing our understanding of the role of ion channels in seizure provides a route to a mechanistic, measurable predictor of seizure. The mechanistic insight gained from characterisation of ion channels involved in seizure would benefit the study of seizure both from both a therapeutic and toxicology screening perspective.

The use of microelectrode arrays (MEA) to monitor spontaneous electrical activity and drug responses in iPSC-derived neuronal cultures may be a suitable method to identify seizure liability *in vitro*.[21] A recent study assessed the acute inhibitory effects of tetrodotoxin in primary rat and human iPSC-derived neuronal networks using MEA with positive results.[22] Unlike calcium imaging utilised by the proposed solution to the CRACK IT challenge, MEA has the potential to monitor the whole electrophysiological profile of the cells. Follow-up patch clamp studies could then provide mechanistic information on which ion channels are responsible for any observed seizurogenic activity. Ion channels determined as possessing a seizure risk could then be transfected into a routinely used cell line and this panel of cells expressing one specific ion channel per cell could be used to screen compounds for seizure risk. This fully integrated screening approach would provide the opportunity to gather mechanistic information in support of optimal drug design early in development to reduce seizurogenic liability. Fig. 2 illustrates where the *in vitro* ion channel seizure panel would fit into the current nonclinical screening paradigm. Although there may still be a requirement for *ex vivo* assays after compounds have been screened on the panel, implementation of the panel would result in a significant reduction in the number of *ex vivo* preparations used, and provides an opportunity for chemical modification following a seizurogenic result.

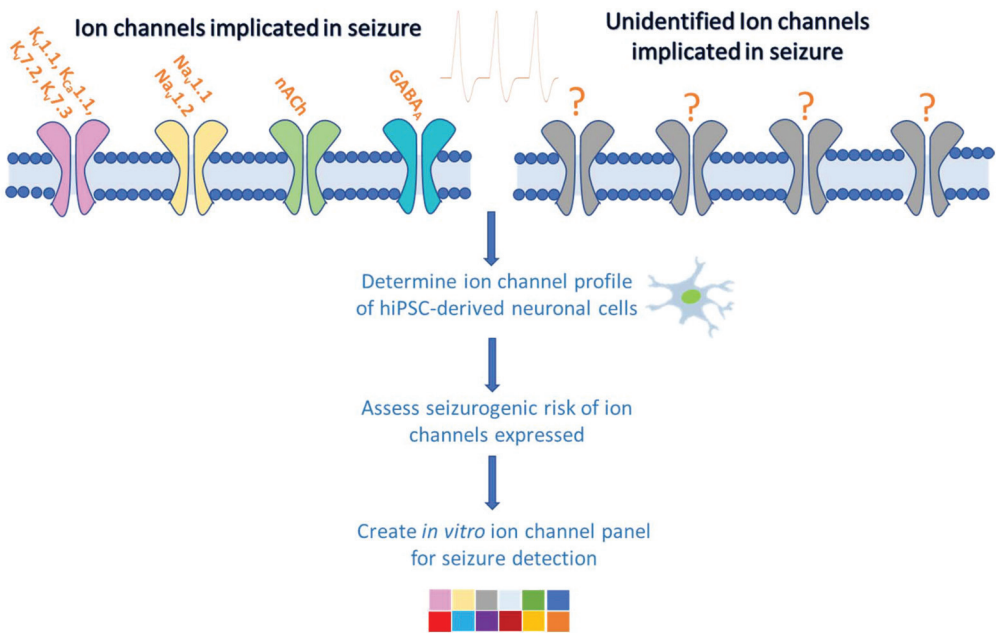

Fig. 1 Development of an ion channel focussed approach to seizure detection. Ion channels in the brain have been associated with seizure (*e.g.* potassium channels, sodium channels, GABA-A and nicotinic acetylcholine receptors),[17,18] however the full complement of ion channels linked to seizure is yet to be resolved. Determination of the ion channel profile of various subtypes of hiPSC-derived neuronal cells followed by assessment of their impact on the seizurogenic phenotype would allow for creation of an *in vitro* ion channel panel for earlier seizure detection. Ion channels determined as possessing a seizure risk could be transfected into a routinely used cell line and this panel of cells expressing one specific ion channel per cell could be used to screen compounds for seizure risk.

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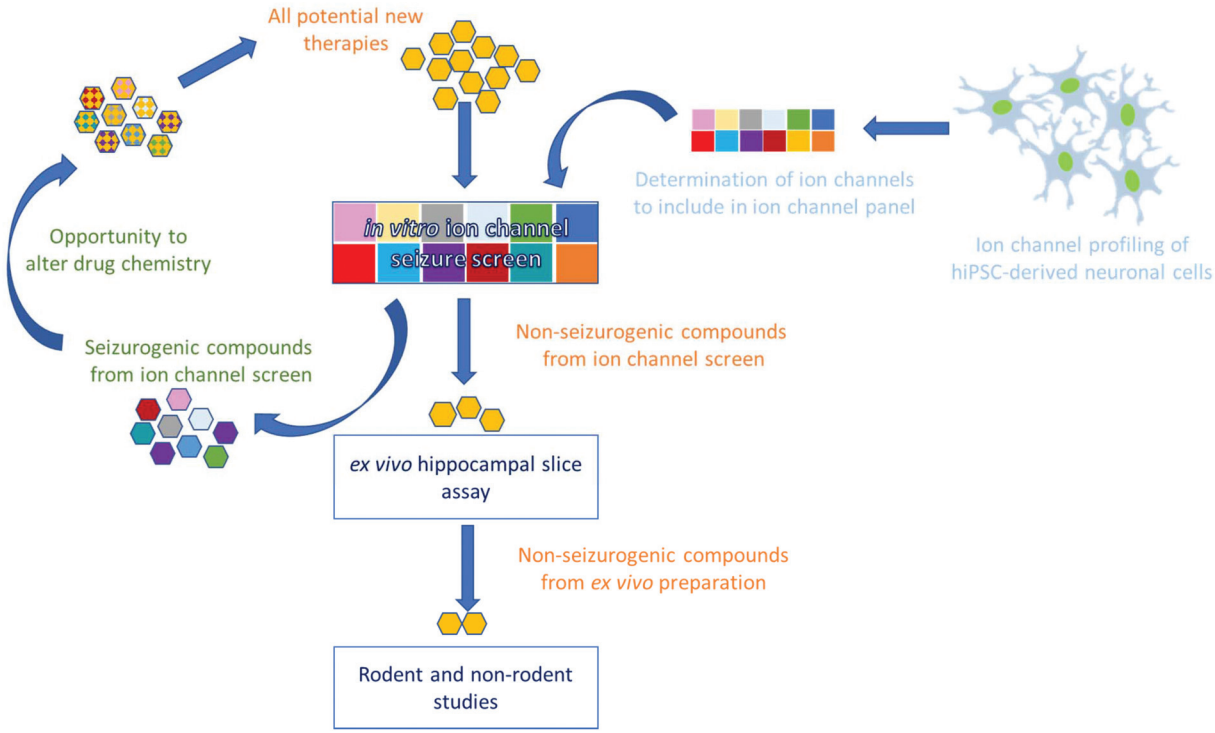

Fig. 2 Potential to improve the current nonclinical screening paradigm for seizure. Characterisation of the ion channels expressed in human iPSC-derived neuronal cells that are linked to seizure and creation of an *in vitro* ion channel panel to screen for seizure could provide a mechanistic approach to nonclinical seizure detection. This approach would be a suitable addition to the existing nonclinical seizure-liability testing strategies that reduces the use of animal and *ex vivo* preparations.

There are multiple advantages to this approach. Nonclinical toxicological assessments should be rapid and reliable with good translatability to humans and ideally use as few animals as possible. The hiPSC-derived cells paired with an MEA-ion channel panel approach has the potential to tick all these boxes. Firstly, ion channels with a seizurogenic risk can be identified in multiple cell types found in the human brain thereby providing physiological relevance, and secondly, MEA allows for real-time, non-invasive, high-throughput analysis of neuronal ionic currents, a method that is both fast and reliable. This approach could be implemented early in drug development to screen out liabilities, before animal testing begins. It may also be interesting to investigate the usefulness of 3D tissue models which may have increased physiological relevance and provide different therapeutic readouts.

The development of innovative *in vitro* screening strategies for seizure liability is an exciting and challenging area of research. Substantial leaps forward in the development and use of human iPSC-derived cells and advancement in research techniques provide the perfect platform to develop innovative translationally relevant screening methods to improve nonclinical screening and reduce the number of seizurogenic compounds that fall out of development during nonclinical toxicology testing or during early clinical development. The current advances in new technologies, such as hiPSC-derived neuronal networks and MEA, provide the opportunity to identify and eliminate new drugs that carry CNS risk earlier and before resources, animals and time have been wasted.

Conflicts of interest

There are no conflicts of interests to declare.

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