



Seizing the moment: Issues and technological advancements in mitigation strategies for drugs with seizure liability

Session Type: Webinar Symposium

Duration: 120 minutes

Chair: Mamta Behl, Ph.D, DABT, Neurocrine Biosciences Inc.

Co-Chair: Chris Strock, Cyprotex

Overview

Despite great progress in our understanding of the structure and functions of the CNS, the discovery of new drugs and their clinical development for many CNS disorders has been problematic. Recent studies have shown that reasons for high attrition rates for CNS drugs are due to two primary reasons: 1) failure in detecting clinical safety and 2) failure in late-stage nonclinical toxicology. Over half of the terminations are for safety liabilities most of which are in animals. While much advancement has been made in exploring in vitro and complementary systems in developmental neurotoxicity and acute neurotoxicity testing, these models are still not as widely applied in routine testing for CNS drugs. This symposium brings together experts in drug discovery and development across the globe to highlight case examples of CNS drug failures during late-stage preclinical toxicology/ first in human (FIH) trials, and sheds light on how some of these novel technologies can be used in lead candidate prioritization and early de-risking.

The chair will briefly introduce the symposium, shedding light on some challenges faced during CNS drug development, and will highlight why animal testing alone may not be sufficient in preventing clinical drug failures. The second speaker from the USA and member of the neurotoxicology HESi group will open the symposium by providing a brief overview of some of the major challenges being faced during CNS drug development, and how in vitro multielectrode arrays are currently being used to predict toxicity during early candidate selection. The third speaker from Japan will provide a case example of how in vitro technology was used to enhance seizure liability prediction in response to a report released by the Ministry of Welfare in Japan about concerns with convulsions in patients following administration of NSAIDs. The fourth speaker from UC Davis will reflect on strategies and challenges for identifying novel and effective interventions for mitigating the onset of seizures triggered by OP nerve agents. Finally, the last speaker from Innovatune, Italy in collaboration with Instem and R3 fellows, USA will shed light on global in silico advancements in the prediction of neurotoxicity including seizure liability with emphasis in current advancements, limitations of current tools, and future goals. This presentation will review the work developed as part of a large expert working group that includes stakeholders from across academia, regulatory agencies, and industry.

The total time for the symposium will be 120 minutes with a 10- minute introduction, 25 minutes per speaker, and 10 minutes at the end for additional questions for the panel.

Speaker 1: Mamta Behl (Chair) (10 min)

Affiliation: Neurocrine Biosciences Inc.

Country: USA

Title: Overview and Introduction

Animal testing is used in pharmaceutical and industrial research to predict human toxicity, and yet analysis suggests that animal models are poor predictors of drug safety in humans especially for the CNS. Today, about 12% of pharmaceuticals pass preclinical testing to enter clinical trials. Of those, only 60% successfully complete phase I trials. Overall, approximately 89% of novel drugs fail human clinical trials, with approximately one-half of those failures due to unanticipated human toxicity. If animal tests accurately predict human toxicity, then why are toxicity-related failure rates in human clinical trials so high and how can the drug development process be improved using complementary models? This talk provides a summary on some current challenges and sets the stage for the subsequent speakers

Speaker 2: Christopher Strock, Ph.D. (Co-Chair) (25 min)

Affiliation: Cyprotex

Country: USA

Email: c.strock@cyprotex.com

Title: Enhancing Seizure Prediction: Rat and Human MEA models offer complementary results

Predicting the seizurogenic and neurotoxic potential of test compounds using microelectrode array (MEA) platforms is an important tool for in vitro neurotoxicity screening. Much of the early work for this involved isolated rat cortical cultures. There have been relatively good responses for rat cortical models in predicting liabilities, but there are certain targets that have not responded well such as muscarinic receptor agonists. Human iPSC-derived glutamatergic neurons co-cultured with human iPSC-derived astrocytes (FCDI) demonstrate a robust maturation process that ultimately forms a highly active and organized neural network. These cells have been effective in identifying liabilities for an overlapping subset of compounds while also being able to identify the muscarinic target that was not identified in rat cortical neurons. However, they fail to identify GABAA antagonists definitively. In further evaluation and comparison of each of these models, it was determined that the response patterns observed for these cells are often complementary.

Speaker 3: Norimasa Miyamoto, Ph.D. (25 min)

Affiliation: Advanced Biological Safety Assessment, Eisai

Country: Japan

Email: n-miyamoto@hbc.eisai.co.jp

Title: Study for Drug-Induced Seizure Prediction Method Using Cultured Neurons and Microelectrode arrays: Rat Primary Neurons vs Rat in vivo Model and Clinical Studies

Drug-induced convulsion is a serious adverse event in clinical. Establishment of in vitro seizure risk assay, which can predict in vivo model's and clinical convulsions, is required for the drug development. In the present study, we are discussing seizure risk assessment potency of an in vitro assay using primary rat cortical neurons and microelectrode arrays (Rat MEA) in comparison with cerebrospinal fluid (CSF) concentrations (conc) induced seizures in the in vivo seizure study. The drug CSF conc in rats with convulsion induced by 6 seizure-positive reference drugs, paroxetine, fluvoxamine, 4-aminopyridine, pentylentetrazole (PTZ), strychnine, and amoxapine, were showing clear convulsion-thresholds. Changes in a parameter, network burst frequency, in the Rat MEA assay was remarkable at seizure-induced CSF conc of the reference drugs, except for fluvoxamine and amoxapine. It is suggested that the Rat MEA assay could be a seizure liability assay for some seizure mechanisms including GABAA receptor antagonism. According to a report to the Ministry of Welfare in Japan, convulsions were induced in seven patients co-administered with enoxacin and fenbufen. Survey result of package inserts and research papers of fluoroquinolones, which were launched in Japan, revealed 3 fluoroquinolones, enoxacin, norfloxacin, and prulifloxacin, are contraindications for coadministration with fenbufen, because of seizure induced possibility. Research results had been published that the fenbufen potentiates in inhibitory effect of fluoroquinolones on the GABA response. Our QPatch GABAA receptor ion channel assay results supported felbinac, a major metabolite of fenbufen, enhanced the GABA antagonism effect of the 3 fluoroquinolones, enoxacin, norfloxacin, and ulifloxacin, an active metabolite of the prulifloxacin, among tested 16 fluoroquinolones. Then, we did Rat MEA assay and in vivo rat tests for cotreated fluoroquinolones and felbinac to compare PTZ case. Then, mechanism of action (MOA) analysis was carried out by principal component analysis (PCA) using multiple MEA parameters. MEA technology potential to predict the seizure liability of drugs with MOA will be discussed.

Speaker 4: Isaac Pessah, Ph.D. (25 min)

Affiliation: UC Davis School of Veterinary Medicine

Country: United States

Email: inpessah@ucdavis.edu

Nicotinic Cholinergic Neurotransmission Is Essential and Sufficient for Induction and Mitigation of DFP-Triggered Electrogenic Seizure Activity In The Hippocampal Slice Preparation

Arguably the greatest challenge for identifying novel and more effective interventions for mitigating the onset of seizures triggered by OP nerve agents has been the lack of in vitro screening platforms. Primary neuron/glia cocultures fail to respond to DFP and nerve agent surrogates despite the fact that they express AChE. Moreover, neurogenic cell lines lack face validity and predictive value. Neurogenic cell models tested to date express functional AChE, muscarinic cholinergic, glutaminergic and GABAergic receptors respond poorly to acute OP exposures and only at high, irrelevant, concentrations. We posited that the inability of dissociated cellular models to assemble and target functional nicotinic cholinergic receptors (nAChRs) to their surface is responsible for their inability to respond OPs. To test our hypothesis, we developed a perforated multi-electrode array approach to measure spontaneous electrical activity (ESA) and AChE activity from acutely cut hippocampal slices from rat and mice. DFP perfusion in aCSF produced time- and concentration-dependent increase in ESA frequency that corresponded with the degree of AChE inhibition in the slice. Perfusion of the pan-AChR antagonist mecamylamine (Mec) 10 min prior to perfusion of DFP was sufficient to significantly reduce seizure-like ESA activity, despite continued exposure to the OP. Timing of Mec with respect to DFP exposures greatly influenced therapeutic efficacy. The $\alpha 4$ -nAChR specific antagonist BHB was sufficient to normalizing seizure-like ESA but the timing of this intervention relative to DFP exposure greatly influenced its efficacy. These results indicate that activation of central nAChRs are necessary and sufficient for eliciting OP-triggering seizure like activity in the hippocampal slice preparation and provides a path forward for screening novel nAChR modulators as potential novel interventions in vitro.

Speaker 5: Arianna Bassan, Ph.D. (25 min)

Affiliation: Innovatune

This presentation will review the work developed as part of a large expert working group that includes stakeholders from across academia, regulatory agencies, and industry.

Member type: non-member

Funding: No funding requested

Country: Italy

Email: arianna.bassan@innovatune.com

Title: Current status and future needs for a neurotoxicity (including seizures) hazard assessment framework that integrates *in silico* approaches

Arianna Bassan¹, Kevin M. Crofton², Glenn Myatt³

¹Innovatune, Via Giulio Zanon 130/D, 35129 Padova, Italy; ²R3Fellows LLC, Durham, North Carolina, 27705, USA; ³Instem, Columbus, Ohio, 43215, USA

The development of more informative new approach methodologies (NAM) to assess neurotoxicity induced by xenobiotics is critically needed to estimate the hazard potential not only for seizuregenic drugs but also for thousands of untested chemicals. The use of NAMs including *in silico* approaches that predict toxicity from chemical structure (e.g., QSARs and structural alerts) is ideally based on an understanding of the biological mechanism underpinning neurotoxicity (e.g., blocking of GABA_A receptors by organochlorines leading to seizures). In this talk, *in silico* methods available today that support the assessment of neurotoxicity based on knowledge of chemical structure will be reviewed, followed by the presentation of a conceptual framework for the integration of *in silico* methods with experimental information. Establishing this framework will be essential for the development of protocols, namely standardized approaches, to ensure assessments of neurotoxicity for chemical structures are generated in a transparent, consistent, and defensible manner.