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Journal of Pharmacological and Toxicological Methods 111 (2021) 107089

Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

journal homepage: www.elsevier.com/locate/jpharmtox

Editorial

Safety pharmacology during the COVID pandemic

A B S T R A C T

This editorial summarizes the content of the current themed issue of *J Pharmacol Toxicol Methods* derived from the 2020 Annual Safety Pharmacology Society (SPS) meeting that was held virtually September 14–17, 2020 due to the ongoing COVID-19 global pandemic. A selection of articles arising from the virtual meeting is summarized. Like previous years they continue to reflect current areas of innovation in SP including new methodologies to predict human safety, best practices for IKr current measurement, and best practice considerations for the conduct of in vivo nonclinical QT studies. The meeting included scientific content from 94 abstracts (reproduced in the current volume of *J Pharmacol Toxicol Methods*). This continued innovation reflects a rubric in SP that identifies problems, seeks solutions and, importantly, validates the solutions.

1. An overview of content from the 2020 SPS meeting

The Annual meeting of the Safety Pharmacology Society (SPS) was held virtually from September 14–17 , 2020. In attendance were 365 registrants representing all aspects of drug safety and pharmacology including those from the pharmaceutical industry, contract research organizations, academia, technology providers and global regulatory agencies. Attendees represented 20 countries and submitted 119 abstracts in total for oral or poster presentations, and there were exhibits from 21 global scientific vendors. The virtual experience for attendees included plenary keynotes, scientific sessions, a networking lounge, poster presentations, as well as scheduled morning and evening social get-togethers. A variety of technology and service providers showcased their latest tools with application to many areas of Safety Pharmacology (SP) within the virtual exhibit hall.

Unlike previous years, in the weeks following the virtual meeting (September 22–October 29) additional content was offered to the attendees on-demand that included continuing education (CE) courses, additional poster discussions, and sponsored sessions. The online CE courses included scientific talks on topics such as the use of artificial intelligence and machine learning in drug development and safety, imaging technologies with applications to exploratory pharmacology and drug safety, the statistical power of SP studies and an overview of intended and unintended effects of drugs on the autonomic nervous system and the downstream impact on key organ systems.

The 2020 Scientific Program featured a broad range of scientific sessions organized into concurrent themed tracks covering issues including:

- Macromolecular therapeutics: cell- and gene-based therapies
- Hemodynamic models; translational approaches and clinical implications
- The value of SP for developing antibody and oligonucleotide therapeutics
- New in vitro and in vivo methodologies to predict human safety
- Predicting clinical safety using animal models
- Covid-19 and cardiovascular function
- ICH E14/S7B update and questions and answers (including discussion on the integrated risk assessment and best practice considerations for in vitro I_{Kr} measurement and in vivo QT studies)

While no distinguished service award recipient was awarded in 2020, SPS provided publication awards for recognition of outstanding scientific research. The technological innovation award was given to Fletcher, Maddock, James, Wallis, and Gharanei (2020) for the publication 'The cardiac work-loop technique: an *in vitro* model for identifying and profiling drug-induced changes in inotropy using rat papillary muscles'. The Translational Safety Pharmacology Award was given to Amouzadeh et al., 2019for the publication 'Clinical implications and translation of an off-target pharmacology profiling hit: adenosine uptake inhibition *in vitro*'.

After the meeting, as has been done since 2004, meeting presentations were developed into manuscripts and submitted for this themed issue, and these are described below. As in previous years they reflect the diversity of SP and the innovation that persists in assay methodology that includes assessment of drug effects on currents as well as novel experimental endpoints that are scientifically valid and robust for use in drug safety studies and a continued modernization of standard assays and preparations. Thus, the pursuit of validation remains avid, and does not appear to be slowing 21 years after the inception of SP as a distinct scientific discipline within the drug safety evaluation spectrum. Most SP method innovation is documented in *J Pharmacol Toxicol Methods*.

Fig. 1 depicts the development of trends in publications that reflects the importance of dynamics in method development in SP since 2004. Articles are primarily 'original articles' that describe and characterize a new or modified model, method, technique, apparatus or approach to analyze data used in the conduct of SP studies. Manuscripts are

https://doi.org/10.1016/j.vascn.2021.107089
Received 14 June 2021; Accepted 18 June 2021
Available online 26 June 2021

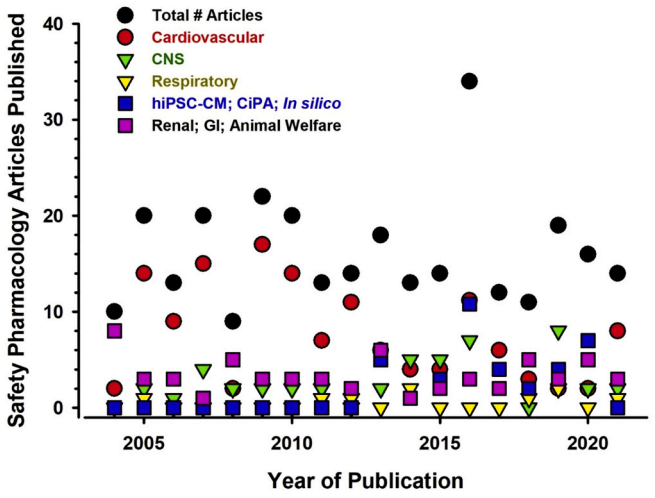

Fig. 1. An overview of publication trends for safety pharmacology manuscripts published in the annual focused issue of the *Journal of Pharmacological & Toxicological Methods*. Included are the total number of articles published each year since 2004 and content. The trends for papers were segregated based upon content and whether it involved core battery studies, i.e., the CNS, CV or respiratory systems. Content is also shown for human induced pluripotent cardiac stem cells (hiPSC-CMs), the Comprehensive in vitro Proarrhythmia Assay (CiPA), in silico modeling methods and Supplemental Safety Pharmacology studies (i.e., Renal or GI studies) surveys and animal welfare studies.

categorized based on the core battery assays, the use of human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM), or in silico modeling methods used in the Comprehensive in vitro Proarrhythmia Assay (CiPA). Lastly, articles related to secondary pharmacological methods (e.g., renal, gastrointestinal) or NC3R practices comprise the other group of regularly submitted manuscripts. This focused issue is devoted to those manuscripts that describe reviews/methods of fundamental importance to SP as well as techniques that are utilized, optimized and validated according to international ethics and scientific quality standards and reflect proper study design, conduct of experiments, data recording methods and data reporting practices. Thus, as it has in the past, this issue will be a primary resource aid to individuals in academia and industry that are interested in understanding SP. While this editorial highlights several key manuscripts included in this issue, readers are encouraged to read all content for state-of-the-art methods across all areas of SP.

2. Cardiovascular safety pharmacology: updates on in vitro safety assays and advanced in vivo methods

2.1. Best practices for automated patch clamp measurements in cardiac safety assessment

Since its inception, SP has evolved methods for the evaluation of New Chemical Entities (NCEs) on cardiac ion channels. The I_{Kr} assay (often referred to as the hERG assay) is conducted as an automated or manual whole cell patch clamp assay. The use of the hERG assay across many different laboratories using similar reference drugs for standardizing channel block, and variable electrophysiological conditions such as different pulse protocols (ramp vs. step), cell lines (heterologous or human), drug perfusion systems or patch clamp method (automated vs. manual) can identify different and sometimes variable IC_{50} values (Blinova et al., 2017; Polak, Wisniowska, & Brandys, 2009). Utilization of automated patch clamp (APC) technology has elevated ion channel research to a high-throughput capacity. The development of high-fidelity recording equipment and innovative cell culture methods has allowed for an advanced electrophysiological assessment of ion channel activity to be conducted in a high throughput manner (Brinkwirth et al., 2020).

In this issue, Bell and Fermini (2021) examine the key moments and drivers in the development of APC technology for ion channel research and provide the reader with an expert opinion on the past and future impact of APC platforms in drug discovery and development. Within just two decades, APC technology had overcome major limitations (e.g., low seal resistance, high cost of consumables) which initially precluded the wide acceptance of APC platforms as an alternative to the "gold-standard" manual patch clamp (MPC) technique. Today, most of the commercially available APC platforms can form giga-Ohm seals, are able to record in current-, voltage- and dynamic-clamp modes, include temperature control, contain microfluidics channels (allowing for fast solution exchange rates and low volume applications) and allow unattended recordings and remote access (Bell & Fermini, 2021). However, while the technical hurdles have been lowered and throughput and quality has been improved, cell performance and reproducibility remain challenging aspects to APC methods (Kramer et al., 2020). There is a clear need to standardize and optimize experimental protocols, recording formats as well as analysis and data export. In that respect, the field is seeking to implement best practices for APC experiments and in vitro studies in general (see also: ICH E14/S7B IWG, 2020).

To outline best practices for conduct of APC measurements of $Na_V1.5$ (the cardiac voltage-dependent sodium channel), Rotordam et al. (2021) studied the effects of multiple experimental variables on IC_{50} values for 24 drugs categorized as high, intermediate, or low proarrhythmic risk in the CiPA paradigm (Gintant, Sager, & Stockbridge, 2016). They evaluated these drugs on $Na_V1.5$ peak and $Na_V1.5$ late currents using the APC platforms SyncroPatch 384 and Patchliner. The authors found that experimental variables such as voltage protocol, recording temperature and compound incubation time affected the potency (IC_{50} value) of test compounds. Interestingly, using frozen vs. cultured cells did not affect drug IC_{50} values, neither did the method of recording $Na_V1.5$ late current (i.e., during the persistent or late (ramp) current of the CiPA step-ramp protocol). Based on their findings Rotordam et al. (2021) recommend the CiPA step-ramp protocol for cardiac safety studies, a physiological recording temperature, and a minimal compound incubation time of five minutes. It is hoped these findings may hasten the routine use of in vitro $Na_V1.5$ peak and $Na_V1.5$ late current assessment in SP.

2.2. Automated blood sampling and the conscious dog QTc assay

The conscious dog QTc assay is used for assessing the potential Torsades de Pointes (TdP) liability of an NCE or biological agent, with inclusion of ancillary variables, by telemetry (Pugsley, Authier, & Curtis, 2008). Variables that are recorded include blood pressure, heart rate and the ECG (PR, QRS and QT/QTc intervals). Since the ECG is dependent on the expression and preponderance of different ion channels it is not consistent between different animal species. The variability may be quantitative (i.e., different currents in the hearts of different species may result in differences in the duration of various ECG segments or intervals (Pugsley, Curtis, & Hayes, 2015)), or qualitative (i.e., the ECG may exhibit a different shape as a result of the expression of an ion channel distinct from that found in the human heart). Minimizing artifacts, such as unwanted noise that may arise during data acquisition, is important to ensure proper interpretation of ECG changes and adequate performance of the automated computerized recording systems (Hamlin, 2005). Many non-cardiovascular study parameters are often recorded with implanted telemetry methods that can contribute to interpretation of the safety data. Since body temperature can affect the QT interval, it is typically recorded (van der Linde et al., 2008).

Cardiovascular studies usually include at least twenty-four hours of baseline monitoring prior to dosing. This duration is necessary to establish the circadian cycle and intrinsic interindividual variability. In addition, cardiac arrhythmias may be detected during ECG interval

assessment. After dosing, a period of twenty-four hours is usually used to monitor effects but may be adjusted based on elimination half-life ($t_{1/2el}$). Thus, prolonged systemic exposure may warrant longer monitoring periods. Drug safety testing strategies should include pharmacokinetic (PK) sampling despite the risk of changes to study variables owing to associated stress, restraint and animal handling. Blood collection in a study has traditionally been optimized to designs that allow sampling without interfering with ECG recordings, which makes it difficult to demonstrate that ECG intervals were evaluated at C_{max}. Safety margins are therefore often dependent on parallel data sets in which the dose obtaining a specified effect is first determined, then administered in a separate study for PK assessment. Incorporation of automated blood sampling (ABS) into a telemetry instrumented canine model may resolve this limitation and provide extra data since it provides for the simultaneous cardiovascular assessment of NCEs and key variables including PK plus blood-based biomarkers, hormones, and cytokines (Koshman et al., 2021). Applying pharmacokinetic/pharmacodynamic (PK/PD) modeling early in the discovery timeline may also allow in vitro EC_{50} values to inform and predict in vivo dose-response of NCEs (Lavé, Caruso, Parrott, & Walz, 2016; Tuntland et al., 2014). In addition, this approach allows examination of the difference between PD time-course and exposure time-course (hysteresis). Traditionally, only parent drug concentrations have been measured and used for safety assessments. However, human drug metabolites may have pharmacological activity (Prasaja, Sasongko, Harahap, & Grigg, 2009; Suzuki et al., 2012), effects on secondary pharmacology (Rothman & Baumann, 2009) and contribute to the adverse drug reaction (ADR) profile (Manyike, Kharasch, Kalhorn, & Slattery, 2000). ABS methodology allows the safety scientist to establish the full PK profile of the parent drug and its metabolites and to investigate the potential contribution of the latter to cardiovascular and electrophysiological ADRs in early exploratory safety assessment without disruption to the telemetry data collection (i.e., the ECG). This approach can also be used to evaluate diurnal and circadian changes in circulating hormones and minimize the impact of animal handling and excitement associated with room entry which is known to affect heart rate and certain hormone measurements (Hopper et al., 2015; Marchant-Forde et al., 2012). ABS allows more robust blood sample data, reduces animal use and improves animal welfare (Schultze et al., 2015). Finally, this methodological approach follows more closely the study design used in the conduct of clinical TQT studies, making ABS studies also more accessible to regulatory authorities who are less familiar with conscious dog nonclinical study design and datasets.

Preclinical strategies for assessing safety liabilities of NCEs vary tremendously between individual companies and are driven by multiple factors including indication (e.g. oncology vs. non-oncology), type of molecule (biologic vs. small molecule) and access to internal and external preclinical capabilities. For example, the use of ABS and CV telemetry recording methods can be combined at several stages during the preclinical drug safety evaluation of an NCE prior to the initiation of GLP SP cardiovascular studies (Koshman et al., 2021). This study type is in accordance with guidance on “best practices” for ICH S7A/S7B CV studies (Leishman et al., 2012) and Koshman et al. (2021) provide a recommendation for incorporation of ABS into in vivo QTc studies. In general, experiments evaluating QTc prolongation using exposure response modeling are statistically more powerful than those utilizing the ‘by-timepoint’ analysis. The combination of ABS with cardiovascular studies in telemetry instrumented dogs may prove invaluable in supporting exposure-response modeling for evaluating the risk of QTc prolongation in nonclinical studies while minimizing the number of animals required for the evaluation, a strategy that supports the expanded NC3R’s goals for reducing animal use based on experiments that are more robust, reproducible and truly add to the knowledge base (Prescott & Lidster, 2017).

3. Biomarkers in safety pharmacology studies

There is a consensus amongst the global regulatory authorities and the pharmaceutical industry that there is a need for standardization of biomarkers. The ideal scenario would be for clinicians to use nonclinical biomarker data to help predict adversity in clinical trials using the same biomarkers (Authier, Pugsley, Troncy, & Curtis, 2013). Previously, the effect of radiation on plasma citrulline levels in different species (mice, Göttingen minipigs and rhesus monkeys) and the effect that experimental study conditions, such as feeding and anesthesia can have on plasma citrulline levels, were examined (Bujold et al. (2016). Plasma L-citrulline is primarily derived from enterocytes in the gastrointestinal (GI) tract from which it is absorbed into the circulatory system. Clinically, L-citrulline is a recognized biomarker of intestinal tissue integrity (Bujold et al., 2016). In this issue, Jäckel et al. (2021) examined the potential role of L-citrulline in toxicology studies conducted in common laboratory animal species for use in the GI safety evaluation of oncology drug candidates. The authors validated a simple bioanalytical method for L-citrulline quantification in plasma. They evaluated L-citrulline in rat and dog repeat-dose toxicity studies and correlated changes to histopathology (i.e., intestinal crypt necrosis, villus atrophy, enterocyte loss) and clinical observations (i.e., bloody feces, diarrhea). L-citrulline levels were decreased after oncology drug treatment in dogs, correlating with toxicological damage to the GI tract. Similarly, L-citrulline levels decreased after oncology drug candidate treatment in rats with increasingly severe changes in histopathology of the GI tract. Interestingly, a $>$ 50% reduction of L-citrulline compared to pre-treatment levels was required before histopathology changes to the GI tract were manifested. L-citrulline therefore represents a potentially relevant biomarker of GI tract integrity that seems to predict GI drug-induced toxicity in advance of histopathological damage. This biomarker may also be used to enable longitudinal evaluation in nonclinical toxicology studies conducted with commonly used species.

Of the core battery of SP methods and study parameters, those related to CV safety have been more difficult to establish than other core battery systems due to a difficulty in reaching consensus about how to test for risk of the rare, but potentially lethal, drug-induced ventricular tachycardia known as TdP. The detection of a preclinical signal for a clinically rare adverse effect is difficult to undertake if the adverse effect is equally rare in animals. When this is the case, surrogate biomarkers are sought; but, surrogate biomarkers for rare events are difficult to validate. The biomarker that has been examined most comprehensively and used most commonly for TdP risk is ventricular repolarization delay (Valentin et al., 2004). This biomarker is used because it has fewer false positives and negatives than other biomarkers such as IC_{50} for blockade of the I_{Kr} current. However, since a delay in ventricular repolarization is not easy to measure, the prolongation of the QT interval is used as a surrogate marker. QT prolongation is the only predictor of TdP that is practicable to record in humans. Therefore, it is sensible to generate data for the same variable in non-clinical safety assessment and test its predictivity. However, more predictive variables, derivable from the ECG, have been sought.

For several years there has been much interest from regulators and SP scientists regarding the J-T_{peak} (defined as the QT interval minus the QRS width) and T_{peak} to T_{end} ($T_{p\text{-}e}$) intervals of the ECG when evaluating drug-induced and congenital proarrhythmic risk (Johannesen et al., 2014; Sugrue et al., 2016). The J-T_{peak} interval represents activity coincident with the phase 2 plateau of the cardiac action potential and data suggests that early repolarization may be a better predictor of drug-induced arrhythmia compared to the QT-interval (Banker, Dizon, & Reiffel, 1997). Today, the standard ECG surrogate for TdP is the duration of the QT interval corrected for heart rate (QTc) (Antzelevitch et al., 2007). However, FDA scientists have shown that the J-T_{peak} interval may be used instead of QTc to discriminate between drugs that inhibit I_{Kr} selectively (e.g., dofetilide) from those that may also block sodium or calcium channels (e.g., quinidine and ranolazine) in humans

(Johannesen et al., 2014).

Skinner et al. (2021) utilized the anesthetized guinea pig and tested an array of selective hERG blockers (with known TdP risk) and drugs with multiple ion channel blocking properties. They asked the question whether J-T_{peak} (corrected for heart rate) and $T_{p\text{-}e}$ (defined the distance between the T-wave peak and return to the isoelectric line of the ECG reflecting transmural dispersion of repolarization) (Hamlin, Kijtawornrat, Keene, & Hamlin, 2003) could be used to differentiate high risk compounds (i.e., dofetilide) from those with a lower propensity (i.e., ranolazine and verapamil) for causing QT prolongation. The authors also evaluated effects of these drugs on the electromechanical window (EMw). The EMw represents the time difference between the end of electrical systole (i.e., the QT interval) and the end of ventricular relaxation (i.e., the LVP_{end} interval) and has been intermittently evaluated as a potential biomarker for TdP risk (Vargas, 2010). In the study, dofetilide was associated with dose dependent increases in QTc, J-T_{peakc} and $T_{p\text{-}e}$. However, ranolazine increased the QTc interval and J-T_{peak} but had no effect on $T_{p\text{-}e}$. Verapamil produced a slight reduction in the QTc interval but only at a high dose (1 mg/kg) with no significant effect on either J-T_{peakc} or $T_{p\text{-}e}$. Only dofetilide produced a dose-related decrease in the EMw. When overall patterns of drug-induced changes in the ECG intervals observed in guinea pigs were compared to clinical data on TdP liability for the same drugs, the predictivity was greatest for dofetilide; however, for ranolazine, although there was a prolongation of the QTc interval in humans and guinea pigs, there was an increase in $T_{p\text{-}e}$ with no change in J-T_{peakc} in humans while in guinea pigs there was an increase in J-T_{peak} and no change in $T_{p\text{-}e}$. At similar free plasma concentrations, verapamil did not produce significant effects on any measured ECG interval in humans or guinea pigs at the doses compared; however, (multi-)channel blockers and additional low-risk compounds should be evaluated. Based upon the current data, the study findings suggest that changes in J-T_{peakC} and $T_{p\text{-}e}$ in guinea pigs do not predict changes in J-T_{peak} and $T_{p\text{-}e}$ in humans.

4. Summary

Each year, SP scientists enhance, adapt and validate non-clinical models for use in early research and development and drug safety assessments. The current series of manuscripts described here reflects this effort. While this editorial addresses some content contained in this SP issue other articles of interest include that by Baldrick (2021) who describes how the current series of core battery safety pharmacology studies need to be overhauled, by van der Linde, Kreir, Teisman, and Gallacher (2021) who propose a nonclinical Beagle dog model to evaluate drug-induced cardiac vulnerability in sudden unexpected death in epilepsy (SUDEP) and by Dodson et al. (2021) from the FDA who evaluated 226 secondary pharmacology profiles obtained from industry sponsors and identify how improvements may be conducted regarding submission of secondary pharmacology studies by pharmaceutical companies. Recently the ICH E14/S7B Q&A guideline (ICH E14/S7B IWG (2020)) has been released by the E14/S7B Implementation Working Group which attempts to outline best practices involved in the design, conduct, analysis, interpretation and reporting of core nonclinical assays and follow-up studies on in vitro hERG block and/or in vivo QTc prolongation. This effort, inspired by the CiPA proposal, addresses the ICH S7B-based “double-negative” nonclinical findings (i.e., low risk for in vitro hERG block and in vivo QTc prolongation at relevant clinical exposure concentrations). Double-negative data has largely been dismissed by the clinical community and does not inform whether or when to undertake a thorough clinical QT study. The aim of ICH E14/S7B Q&A guideline is to assist clinical decision making by addressing models, key experimental variables, and data quality focused on the interpretation of clinical QTc prolongation and associated proarrhythmia risk. (Vargas et al., 2021). This will be particularly useful when clinical TdP liability evaluations cannot be conducted owing to low drug exposures or high-dose safety issues (Vargas et al., 2021).

Disclaimer

The opinions presented here are those of the authors. No official support or endorsement by participating companies is intended or should be inferred.

Declaration of Competing Interest

None of the authors have any conflicts of interest, other than employment at either a contract research organization (SA), a biotech company (TdeK) or a pharmaceutical company (MKP, YK).

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