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COMMENTARY

International Life Sciences Institute (Health and Environmental Sciences Institute, HESI) initiative on moving towards better predictors of drug-induced torsades de pointes

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Knowledge of the cardiac safety of emerging new drugs is an important aspect of assuring the expeditious advancement of the best candidates targeted at unmet medical needs while also assuring the safety of clinical trial subjects or patients. Present methodologies for assessing drug-induced torsades de pointes (TdP) are woefully inadequate in terms of their specificity to select pharmaceutical agents, which are human arrhythmia toxicants. Thus, the critical challenge in the pharmaceutical industry today is to identify experimental models, composite strategies, or biomarkers of cardiac risk that can distinguish a drug, which prolongs cardiac ventricular repolarization, but is not proarrhythmic, from one that prolongs the QT interval and leads to TdP. To that end, the HESI Proarrhythmia Models Project Committee recognized that there was little practical understanding of the relationship between drug effects on cardiac ventricular repolarization and the rare clinical event of TdP. It was on that basis that a workshop was convened in Virginia, USA at which four topics were introduced by invited subject matter experts in the following fields: Molecular and Cellular Biology Underlying TdP, Dynamics of Periodicity, Models of TdP Proarrhythmia, and Key Considerations for Demonstrating Utility of Pre-Clinical Models. Contained in this special issue of the *British Journal of Pharmacology* are reports from each of the presenters that set out the background and key areas of discussion in each of these topic areas. Based on this information, the scientific community is encouraged to consider the ideas advanced in this workshop and to contribute to these important areas of investigations over the next several years.
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Abbreviations: APD, action potential duration; EADs, early afterdepolarizations; ECG, electrocardiograph; hERG, human ether-à-go-go related gene; I_{Kr}, rapid delayed rectifier (potassium) current; LQTS, long QT syndrome; NME, new molecular entity; TdP, torsades de pointes

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Introduction

Knowledge of the cardiac safety of emerging new drugs is an important aspect of assuring the expeditious advancement of

the best candidates targeted at unmet medical needs while assuring the safety of clinical trial subjects or patients. Present methodologies for assessing drug-induced torsades de pointes (TdP) are woefully inadequate in terms of their specificity to identify pharmaceutical agents, which are human arrhythmia toxicants. This concern reflects the state of science in understanding the mechanisms of drug-induced polymorphic ventricular tachycardia or TdP. TdP is an extremely rare event with non-antiarrhythmic drugs (Darpo, 2001) with an incidence of arrhythmia as low as 1–4 in 100 000 or less (Wysowski and Bacsanyi, 1996; Camm, 2005). As a result, TdP has either gone undetected or has not occurred in numerous pre-clinical and clinical trials. This has forced investigators to rely on alternative indices, different *in vitro* and *in vivo* models of a delayed cardiac ventricular repolarization, or QT interval prolongation, as surrogate biomarkers of TdP risk.

One difficulty with these pre-clinical and clinical indices of prolonged cardiac repolarization as surrogate markers of TdP is the low specificity of the assays and thus the potential for promising new test agents to generate false-positive results with a high frequency. In addition, the fact remains that the incidence of TdP, which is the true risk associated with pharmaceutical treatments, is seen significantly less frequently than QT interval prolongation. Alternatively, there are examples where the absence of QT interval prolongation in preclinical models did not predict the presence of QT interval prolongation in clinical studies (FDA, 2003). Importantly, clinical studies of new therapeutic agents suggest that QT interval prolongation alone does not necessarily lead to proarrhythmias (FDA, 2003). Thus, the critical challenge in the pharmaceutical industry today is to identify experimental models, composite strategies, or other biomarkers of cardiac risk which can distinguish a drug that prolongs cardiac ventricular repolarization, but is NOT proarrhythmic, from the one that prolongs QT interval and leads to TdP.

To that end, the Proarrhythmia Models Projects Committee of the International Life Sciences Institute (Health and Environmental Sciences Institute, HESI) recognized that there was little practical understanding of the relationship between drug effects on cardiac ventricular repolarization and the rare clinical event of TdP. It was on this basis that a workshop 'Moving Towards Better Predictors of Drug-Induced Torsade de Pointe (TdP)' was convened in Crystal City, VA, USA in November of 2005.

The primary objective of the workshop was to develop a better fundamental understanding of the emerging science, trends and methods and methodologies that relate to predicting drug-induced TdP. Specifically, the objectives were to:

(1) 'Identify the underlying (known or novel) mechanisms for drug-induced TdP arrhythmia to develop better tools for identifying drugs at risk;
(2) Evaluate and assess emerging pre-clinical methodologies for predicting drug-induced TdP;
(3) Identify biomarkers in pre-clinical studies that may be applied to clinical testing for drug-induced arrhythmia;
(4) Identify the critical aspects of pre-clinical and clinical methods of evaluating potential for drug-induced TdP in the context of public health decision-making; and
(5) Identify short and long-term priorities for developing better predictors for drug-induced TdP.

The first day of the 2-day workshop was devoted to a series of presentations by experts in the field of cardiovascular research and safety assessment. The reader is referred to other sections of this special issue of the *British Journal of Pharmacology* for a review of current knowledge in the selected areas as presented by these speakers (Bass *et al*., 2008; Fossa, 2008; Lawrence *et al*., 2008; Pollard *et al*., 2008; Roden, 2008; Sager, 2008; Salama *et al*., 2008; Sugiyama, 2008; Vos, 2008).

On the second day of the meeting, four breakout sessions were convened. Assignment of meeting participants to the breakout sessions assured a thorough consideration of each topic. The four topics discussed included: Molecular and Cellular Biology Underlying TdP (Session 1, Co-Chairs: Craig January and Dan Roden, Rapporteurs: Ying-Ying Zhou and Kristy Bruse); Dynamics of Periodicity (Session 2, Chair: Derek Leishman, Rapporteurs: Jean-Pierre Valentin and Dianne Garnes); Models of TdP Proarrhythmia (Session III, Co-Chairs: Wilhelm Haverkamp and Marc Vos, Rapporteurs: Hal Feldman, Alexander Breidenbach and Chris Lawrence); and Key Considerations for Demonstrating Utility of Pre-Clinical Models (Session IV, Co-Chairs: Borje Darpo and John Koerner, Rapporteurs: Philip Sager and Tim Hammond).

Along with the speakers from day 1, the chairs of each session described the state of knowledge in the topic area and the main areas of consensus and debate. This publication captures the key points from this 2005 workshop along with discussions and recommendations reflective of the state of science in each of the subject areas in 2008. Recommendations for further study are provided here, but the reader should consider them in light of their own knowledge of the molecular and cellular mechanisms that may underlie TdP and their own view of research that will shed further light and focus on the overall goal of identifying better predictors of drug-induced TdP.

Molecular and cellular biology underlying TdP

Introduction

This section is focused on the evaluation of the fundamental molecular and cellular biology underlying TdP and identifies areas to develop better predictors with emerging science and technologies. A better understanding of the fundamental molecular and cellular biology underlying TdP will help scientists develop pre-clinical assays with high sensitivity and specificity for TdP. For the background that follows, the reader is also directed to the publications that appear in this special issue by Roden, 2008.

Variables in $I_{Kr} \rightarrow APD \rightarrow QT \rightarrow TdP$ relationship

The most common practice in cardiovascular safety pharmacology is to assess the risk of delayed ventricular repolarization and TdP as recommended by the ICH S7B guideline (ICH Harmonized Tripartite Guideline S7B, 2005). This may be achieved *in vitro* by evaluating the function of the I_{Kr} (I_{Kr} equivalent to Kv 11.1 (Alexander *et al*., 2007)) and the rapid

delayed rectifier (potassium) current or *in vivo* with an electrocardiograph (ECG) QT interval assessment that represents an integrated information of all channel currents, including $I_{Kr,}$ during the time course of ventricular repolarization. Additional assays, such as a repolarization assay in isolated tissue (for example, canine Purkinje fibre or guinea pig papillary muscle), may be useful if needed to further elucidate a mechanism of action or clarify potential risk. The first part of the discussion centres on the relationship between I_{Kr} inhibition, increased action potential duration (APD) and/or early afterdepolarizations (EADs) to the risk of drug-induced QT prolongation and TdP.

The I_{Kr} assay has been established in the pharmaceutical industry for almost a decade (Trudeau *et al*., 1995; Mohammad *et al*., 1997; Rampe *et al*., 1997), but, the question of how to quantify the drug-induced I_{Kr} block remains a critical issue (Redfern *et al*., 2003). There are several factors that vary across laboratories and can significantly influence the *in vitro* I_{Kr} results and which therefore may underlie between-laboratory variation in IC_{50} values (Kirsch *et al*., 2004; Hanson *et al*., 2006). These factors include, but are not limited to, the electrophysiology protocol, experimental preparations and conditions including cell type, solutions and temperature, drug adsorption to the perfusion tubing and other apparatus in the testing system. Despite identical protocols and experimental conditions, a general threefold or greater difference between two laboratories was observed in the I_{Kr} (human ether-à-go-go related gene, hERG) assay conducted as prospective pre-clinical studies in a prior initiative of the HESI Cardiovascular Safety Projects Committee (Hanson *et al*., 2006).

The kinetics of the I_{Kr} current, the state- or use-dependency of the block, the ancillary subunits (MiRP1, mink) and the intrinsic drug properties, though not evaluated as a routine, could also impact the interpretation and translation of the *in vitro* I_{Kr} data to predict the clinical outcome (Spector *et al*., 1996; Anantharam and Abbott, 2005). Furthermore, the polymorphism in the patient population, the potential for accumulation of the drug in cardiac tissue and the calculation of the degree or percentage of protein binding into this complex equation makes the risk assessment process even more complicated (Redfern *et al*., 2003; Titier *et al*., 2005; Hoffmann and Warner, 2006; Modell and Lehmann, 2006). Rather than seeking a perfect model at this moment, the recommendation for future practice is to minimize between laboratory variations by harmonizing test systems, standardizing the verbiage and normalizing the data (for example, use relative potency against a positive control or target pharmacophore).

Even though I_{Kr} blockade was recognized as the most common mechanism of APD or QT prolongation induced by pharmaceuticals, other mechanisms may significantly cause APD prolongation (for example, I_{Ks} blockade) and/or shape the final outcome of action potential or QT interval. For example, targets such as other inward and/or outward currents could be 'protective' against I_{Kr} blockade. When ion channels other than I_{Kr} should be evaluated and whether *in silico* modeling could assist in simulation of other ion current results requires further investigation. An APD assay, such as the Purkinje fibre assay, is very useful to identify effects from multiple channels (Martin *et al*., 2004), but the sensitivity of the assay for various preparations (dog, rabbit, and so on) and the choices of the parameters (for example, APD, triangulation, reverse-use dependency and instability) are still under examination (Brown, 2005; Hanson *et al*., 2006; Lawrence *et al*., 2006; Thomsen *et al*., 2006). An appropriate positive control reference compound should always be included to demonstrate the sensitivity of any APD assay. More recently, altered channel trafficking has emerged as an alternate mechanism for I_{Kr} reduction (Eckhardt *et al*., 2005). The importance of this mechanism in the acquired long QT syndrome (LQTS) needs to be further assessed. Finally, over-interpretation of the I_{Kr} blockade data should be avoided and the strengths and weaknesses of each alternative complimentary assay should be considered.

Although there is no agreement as to the electrophysiological 'shape' of an EAD, the consensus is that EADs can cause dispersion of repolarization and/or trigger activity which leads to TdP. There are more questions than answers regarding how much of an increase in APD and/or the amplitude of the EAD actually perturbs the QT duration or produces electrocardiogram morphology changes (for example, TdP). Under what conditions is QT prolongation beneficial (that is antiarrhythmic properties) and under what conditions does it constitute a risk? Are there a constellation of properties that distinguishes a drug that is not proarrhythmic from one that is proarrhythmic, despite causing similar degrees of QT prolongation? These questions serve as the basis for much of the ongoing research in the cardiovascular proarrhythmia safety community.

Evolving tools to move to better predictors of drug-induced TdP

It is critical to the field to assess the evolving tools that are or could be made available to make drug-induced TdP more predictable. One useful tool is *in silico* modeling, which includes ligand-based modeling (for example, pharmacophore modeling), target-based modeling (for example, structure modeling of hERG and other ion channels) and electrophysiology modeling for a single cell or even whole heart. Pharmacophore modeling and ion channel structure modeling can refine the chemical structure design for future *in vitro* testing and serve as rank ordering tools in early discovery where the objective is selection of candidate chemical templates (Aronov, 2005). At a higher level of integration, the modeling of the cardiac action potential or even the whole heart will allow testing of hypotheses otherwise not easily accessible to a high throughput strategy (Kleber and Rudy, 2004). However, for these to be accomplished extensive validation is required to prove the reliability of these computer-based modeling approaches.

The future for *in vitro* biology of I_{Kr} channel inhibition should be focused towards a better understanding of the regulation and dynamics of the channel, including the lipid and structural influences, subunits and other interacting proteins, transcriptional and post-transcriptional regulation and the post-translational processing. Other factors such as adrenergic tone and magnesium and potassium concentrations can elicit direct or indirect effects on the I_{Kr} current.

With this advanced knowledge, the manipulation of the I_{Kr} channel at the cellular level, the organ level, and even the animal level could possibly become a powerful tool for TdP prediction. The 'cutting edge' science of stem cell research and transgenic non-rodent animal models might bring novel tools for drug safety evaluation of TdP, though these deliverables are not expected within the next 5 years.

The importance of the altered intracellular calcium dynamics and subsequent activation of downstream signal transduction pathways (for example, Ca^{2+}/calmodulin-dependent protein kinase II) are emerging as key elements in the field of cardiac safety. Calcium is postulated to underlie the generation of EADs and prolongation of the action potential leads to an increase in the intracellular calcium levels and activates Ca2 + /calmodulin-dependent protein kinase II (Anderson, 2006). Certainly, direct high throughput screening of drug effects on intracellular calcium transient, action potential, and arrhythmogenicity of mammalian and human myocytes will be encouraged.

Last and most importantly, there is a need for global genetic screening and a search for relevant biomarkers. Concerted and collaborative efforts from academia, industry and regulatory agencies are required to ascertain DNA samples, ECG recordings and clinical data from a large number of patients, including those with drug-induced TdP. These efforts will assist in the development of a platform that could foster discovery and characterization of the sequence variant in the patient population (Roden, 2006). The understanding of the role of genetic variants will help identify patients at risk for TdP, which would contribute to tailoring of therapeutic drugs to the various patient populations in the future. Additionally, understanding the basis for greater susceptibility may also point to unique mechanisms, which could explain the propensity of a complex series of events to elicit arrhythmia. This would further provide avenues for modification of test substances that may be impacting these mechanisms.

Conclusion and recommendations

In summary, there are a lot of variables that remains to be explored, in addition to the simple formulation of $I_{Kr} \rightarrow APD \rightarrow QT \rightarrow TdP$. The recommendation is to continue the effort to standardize the I_{Kr} assay and further understand kinetics and regulation of I_{Kr}. Additional variables, including information from other cardiac ion channels or changes in the pattern and dynamics of the action potential and the ECG (as described elsewhere in this special issue of the *British Journal of Pharmacology*: Lawrence *et al*., 2008; Pollard *et al*., 2008; Salama *et al*., 2008; Sugiyama, 2008) might add value to the integrated risk assessment. We should also take advantage of the *in silico* modeling, recent progress in *in vitro* cell biology, incoming stem cell and transgenic non-rodent animal models and other evolving tools to advance our knowledge and techniques in this area. Research on the genetic variants and biomarkers will also enhance the understanding of the relevant mechanisms as well as provide direction for developing better biomarkers and treatment strategy.

Dynamics of periodicity

Introduction

Much of the biological data collected during the evaluation of cardiac repolarization exist as a time series. End points such as QT interval are potentially available for consecutive beats over considerable periods of time. In conventional analyses of QT interval, however, much of the time sequence data which are available is not utilized. Time is primarily used when time-matching across treatment groups to control variables such as diurnal variation and specific or spontaneous events affecting all groups. The relationships between consecutive beats are lost when examining a single beat, averaging a number of beats, or in conducting 'Holter bin' type analyses. In contrast there are examples of time series analysis adding value in evaluations of the cardiovascular system, such as heart rate variability in assessing autonomic function. Spectral analysis is often used to assess heart rate variability; however, this technique focuses on discrete periods of time and ignores large amounts of data which could be included in sophisticated non-stationary analysis techniques (Humphrey and McCall, 1982; Bedford and Dormer, 1988; Mangin *et al*., 1998; Fossa *et al*., 2002). This discussion is an attempt to capture some of the information and experience that is emerging from analyses of the time series nature of QT and heart rate data. In addition to the text that follows, the reader is also directed to the publications which appear in this special issue by (Fossa, 2008; Lawrence *et al*., 2008; Pollard *et al*., 2008; Salama *et al*., 2008).

Discussion

The underlying mechanism of drug-induced TdP is probably multifactorial and it seems inappropriate to rely solely on a fixed index such as an average QT prolongation in representing this complex, dynamic pathophysiological event. By focusing on mean QT interval only, we are not capturing potentially valuable information on QT dynamicity, beat-to-beat restitution (QT–TQ relationship) and beat-to-beat hysteresis, which may substantially improve the predictive value of the assay (Salama *et al*., 2008).

There are several examples where time series data could be considered in terms of the overall shape of the QT and RR or heart rate 'cloud' at discrete time points before and during drug treatment (Fossa *et al*., 2002, 2005, 2006). This type of analysis could make more utility of the time series nature of the data when considering the interval between the beats as well as the QT interval—by examining QT vs TQ (Fossa *et al*., 2006). This latter approach is an examination of QT restitution and examples in which significant beat-to-beat variability were correlated with proarrhythmias can be explored. A strong influence of sympathetic nervous system activity, a known contributor to proarrhythmia, was also illustrated in this QT vs TQ analysis. In the guinea pig, measurement of monophasic action potential duration alternans and cardiac instability has been successfully used

to differentiate the proarrhythmic profile of antibiotics (Wisialowski *et al*., 2006). Another technique, short-term variability in the chronically AV-blocked dog to examine the dynamic nature of the QT interval can also be explored (Volders *et al*., 2003; Thomsen *et al*., 2006). This technique has demonstrated some utility in separating QT prolonging agents associated with TdP from those generally believed to be less proarrhythmic and/or to separate the dose–response relationship on QT vs TdP. This *in vivo* analysis used the same Poincaré plots that have been proposed for an *in vitro* rabbit model of proarrhythmia (Hondeghem *et al*., 2001; Valentin *et al*., 2004). This *in vitro* demonstration of instability is a key determinant of proarrhythmic potential in that model.

Investigations into the dynamics of repolarization are at present rare and the techniques and proprietary software for such analyses are not widely available. However, a number of key questions should be addressed. First, what is the best way to succinctly describe the dynamics of repolarization? Second, what are the relevant species to be examined? Test species should be selected based on their relevance to man with regard to ion channel composition, cardiac action potential morphology as well as the nature and control of the dynamics of repolarization. Ideally a model system would be one in which TdP could be elicited to probe the dynamic patterns leading to the onset of arrhythmia. Should the animals be naive or modified to model pathological conditions relevant to arrhythmia? In addition, wider aspects of repolarization such as alternations in T-wave morphology should be considered from a dynamic view.

Recommendations

Analysis of repolarization dynamics may be a means for separating proarrhythmic drugs from those that are non-proarrhythmic, despite QT prolonging effects. As we can already determine whether or not a compound affects cardiac ventricular repolarization, the challenge is the ability to distinguish between drugs that are mild QT prolongers (5–10 ms) without proarrhythmic hazard and drugs that are potentially harmful.

Although there are daunting questions around the application of analysis of the dynamics of repolarization, there are some simple experiments one might consider to supplement the encouraging data with dynamic preclinical models of proarrhythmia. The recommendation is to conduct a series of studies to collect beat-to-beat QT and RR interval data in key species and models. These models could include naive monkey, dog, guinea pig and rabbit as well as AV-blocked dogs and monkeys (Sasaki *et al*., 2005; Schneider *et al*., 2005; van der Linde *et al*., 2005; Takahara *et al*., 2006; Thomsen *et al*., 2006; Wisialowski *et al*., 2006). *In vitro* models (for example, cardiac myocytes, Purkinje fibre, isolated heart) can also be considered although they may lack some external influences on dynamics which could be important (Hondeghem *et al*., 2001; Lawrence *et al*., 2005; Lu *et al*., 2006; Wu *et al*., 2006). The availability of beat-to-beat data would allow a range of analysis types to be tested. In those species/models showing promise, key compounds should be tested (that is, compounds covering a broad spectrum of torsadogenic propensity) (Redfern *et al*., 2003; Lawrence *et al*., 2006; the reader is also referred to the section 'Key considerations for demonstrating the utility of pre-clinical models' in this publication and the publication by Sager, 2008 in this special issue).

Conclusion

Encouraging emerging reports suggest that taking advantage of the dynamic aspects of collected QT interval data may provide an improved means of predicting which drugs are potentially proarrhythmic. Although this is an aspect of cardiovascular evaluation that is currently very specialized and in its infancy there are some simple steps which can be taken to explore this further. Simply put, we should collect beat-to-beat data in species/models, which appear promising and with key validation compounds and make this data available to groups with experience in this area of study. This would provide a core resource to help identify the dynamic characteristics, which are most predictive of TdP. Ultimately such markers of drug-induced TdP could be applied clinically.

Models of TdP pro-arrhythmia

Introduction

An introduction to the current experience and knowledge of existing proarrhythmia models and parameters from which to investigate drug-induced TdP appears in this section and elsewhere in this special issue of the *British Journal of Pharmacology* (Lawrence *et al*., 2008; Pollard *et al*., 2008; Sugiyama, 2008; Vos, 2008). The suggested testing battery in the current ICH S7B guidance (ICH Harmonized Tripartite Guideline S7B, 2005) (*in vitro* I_{Kr} and *in vivo* conscious non-rodent telemetry), though adequate for predicting the risk of QT interval prolongation in humans, is unlikely to generate sufficient data to identify compounds with a potential torsadogenic risk. Although the incidence of drug-induced QT interval prolongation is not uncommon, torsades de pointes is a rare event in humans. Any proarrhythmia model needs to be capable of reliably reproducing this arrhythmia. Our challenge is to understand the relationship between animal models and human experience. The following topics are discussed: (i) models of proarrhythmia (ii) parameters that can determine a compound's proarrhythmic liability, (iii) a proposed validation strategy for determining the predictive value of the various models and parameters.

The models

General characteristics of an acceptable proarrhythmia model include high reproducibility, specificity and sensitivity. There is an agreement that the primary goals of any adopted models are that they should be capable of minimizing the number of false-negative results and identifying false-positives in the primary *in vitro* and *in vivo* assays. It is also critically important that such models be capable of identifying those drugs that are not classically considered to be QTc-prolonging molecules. For example, I_{Ks} blockers that

are devoid of proarrhythmic propensity at resting heart rates, may prove to be torsadogenic at faster heart rates (during exercise) where there is no concomitant decrease in the QT interval. Potential models must be capable of identifying such proarrhythmic compounds.

Sensitivity and specificity. Sensitivity and specificity are important considerations, although it is admitted that acceptable levels for proarrhythmia models have yet to be agreed upon. This is a primary issue that needs to be defined as early as possible in the process of identifying an acceptable strategy. Owing to biological variability, it is not reasonable to expect any proarrhythmia model to be 100% predictive. As proarrhythmia assays often will be follow-up tests, in many cases to further study a positive signal in a basic screen such as hERG with a high proportion of false positives, one of the most important features of the test must be to identify true positives.

The concern that proarrhythmia models may be too sensitive could be balanced by the argument that an increase in specificity could help resolve such potential sensitivity problems. Alternatively, it may be possible to use information from highly sensitive models, such as those based on deficiency in I_{Kr}, to design drug treatment plans for patients with certain underlying pathology, which can exclude particular drugs. For this patient type, this is exactly the type of screen that may be needed. However, this offers little comfort for the patient with a silent mutation that may become unmasked with drug treatment. Identifying the risk for this patient type is complicated by the limited knowledge about the mutations causing acquired LQTS and congenital LQTS. Furthermore, certain pathological conditions (for example, cardiac disease) might contribute to relative patient risk.

The role of proarrhythmia models. The value of proarrhythmia models is in determining the potential of compounds to induce TdP when there is a positive signal in other QT-related models (particularly for potential drugs being developed in areas of unmet medical need). In other words, proarrhythmia models should help to identify whether a drug, despite an unwanted electrophysiologic profile including I_{Kr}-block and/or APD/QT/QTc prolongation, has an inherent propensity to elicit TdP. The successful registration of ranolazine is a case in point: even though ranolazine shows dose-dependent APD/QT/QTc interval prolongation it was clearly not proarrhythmic in pre-clinical models—indeed ranolazine abolishes cisapride-induced EADs and ectopic beats (Antzelevitch *et al*., 2004a, b; Schram *et al*., 2004; Singh and Wadhani, 2004; Song *et al*., 2004). It is recognized that TdP proarrhythmia models were important in supporting the late stage development of ranolazine leading to approval of the drug, even though strict labeling limitations were applied and the post-approval clinical experience is still limited.

Owing to the complexity of identifying the proarrhythmic potential of candidate drugs it can be argued that more than a single model or parameter may be required; compounds could be tested in highly susceptible models of increasing complexity from isolated tissue and/or heart to whole animal. The final level of complexity would be an intact 'normal' animal followed by proarrhythmia-prone disease entities, for example, the ischemic heart or the infarcted heart.

In vitro *models*. *In vitro* models have the potential to offer greater experimental control and flexibility compared with *in vivo* models. For example, using isolated tissues or isolated heart preparations allows certain clinical conditions to be mimicked, for example, hypoxia, ischaemia, metabolic disturbances or electrolyte changes. *In vitro* models also allow a greater possibility to explore potential underlying arrhythmogenic mechanisms. If there is indication from the hERG assay that repolarization could be delayed, but *in vitro* models of proarrhythmia show negative results, mechanistic follow-up studies (effects on other cardiac ion channels) may be necessary to elucidate the discrepancy. This particular scenario highlights the importance of an integrated cardiovascular risk assessment.

In vivo *models*. It is not clear whether a complete *in vitro* and *in vivo* data set is necessary for each new compound. It could be suggested that there is a need to employ *in vivo* models to qualify positive findings in *in vitro* assays. This leads to the strong suggestion that an *in vivo* model should be sufficiently sensitive to reliably reproduce TdP, for example, chronic AV block or cardiac hypertrophy.

Selection of pathological models. Pathological models may play an important role as tools to represent subsets of the population with pre-existing and/or concomitant conditions, for example, ischemic heart disease, who may be particularly vulnerable to drug-induced proarrhythmia. The question is whether proarrhythmia models should include any scenario that in a particular patient population might be prevalent? It is not a realistic expectation to provide proarrhythmia models for every possible concomitant condition, for example, diabetes, heart failure, renal impairment, and so on; the need for such extensive disease models can be questioned. It is suggested that a testing scenario of sufficiently high sensitivity might help to lessen concern regarding preexisting conditions that may increase the risk of proarrhythmia.

Properties of a TdP proarrhythmia model. Recommendations for the properties of a TdP proarrhythmia model should include:

(1) Model should provide adequate testing throughput to meet the users needs: (for example, *in vitro* throughput comparable to manual patch-clamp techniques and *in vivo* throughput comparable to non-rodent telemetry studies);
(2) Intact animal models should employ a conscious animal;
(3) Animal models should reliably develop TdP when challenged by a known torsadogenic compound (high specificity); and
(4) The model should have high sensitivity at the clinically therapeutic dose and beyond.

The overall goal is to obtain a profile of the safety-relevant properties of a drug. This approach would enable the knowledgeable scientist to construct an overall integrated risk assessment and make decisions regarding the future of a particular compound.

The parameters

It is widely accepted that a number of parameters exist from which to define a compound's proarrhythmic potential (Thomsen *et al*., 2006). Parameters of importance include arrhythmias, EADs, spatial and temporal dispersion, local and global repolarization times and reverse frequency-dependence (the reader is also referred to the sections, 'Molecular and cellular biology underlying TdP' and 'Dynamics of periodicity' in this publication). This is a non-exhaustive list; however, these parameters are most often noted as of primary importance. It is also possible that these parameters may need to be reconsidered if new insights into mechanisms of TdP are revealed. The question of whether one model should comprise all parameters of interest or whether individual parameters are to be investigated in different models should also be considered. There is likely to be some overlap of parameters between models.

Recommendations: a proposed validation strategy

There should be a uniform approach within the pharmaceutical industry worldwide to develop a package utilizing the same models, protocols and parameters, objectively analyzing the results and developing a consensus opinion on the value of TdP proarrhythmia models.

Drug set. In general, any validation efforts are dependent on a set of positive and negative control drugs that will be used for a validation programme and for which a wide agreement, including regulatory authorities, academia and pharmaceutical industry, must exist. For further discussion on characteristics of such drugs, refer to the section, 'Key considerations for demonstrating the utility of pre-clinical models'.

Models and parameters. The selected drugs would be tested in a number of models in different laboratories and analysed by different groups under blinded conditions. The ultimate aim is to test the ability of the model/parameters to identify proarrhythmic signals (yet to be clearly defined). An approach could be to start a pilot study, simply with three drugs, one from each category (positive, that is, positive I_{Kr} and clinical evidence of TdP; questionable: that is positive I_{Kr} and no TdP; and negative: that is, negative I_{Kr} and no TdP) and then refine the study as appropriate. Any validation process should involve a multicentre approach. Criteria for interpretation and control of multi-site variability should be defined prior to the start of the validation.

Species selection. The New Zealand white rabbit is considered to have clear advantages as a model for proarrhythmia. Several *in vitro* and *in vivo* proarrhythmia models employ the rabbit, because of its reduced expression of I_{Ks} in the heart and therefore its inherent susceptibility to TdP arrhythmias. In contrast, the dog is limited to use in a smaller subset of models. For example, a genetically modified heterozygous rabbit model exists which is deficient in I_{Kr}. Albeit not generally accessible at the moment, it is a promising model for future considerations. Clearly the characteristics of any proarrhythmia model need to be known and the implications fully understood as drugs might elicit arrhythmias that are not clinically relevant.

Several species need to be included in a validation process. Non-human primates (NHP) may be considered in cases where the metabolic profile of a compound in humans is more appropriately reflected in this species rather than in dogs or rabbits (Sugiyama, 2008). NHP may also be important where the compound is targeted at a site not expressed in other species or where mechanism-based effects are possible. Other alternative approaches may include testing of the active metabolite separate from the parent compound.

Conclusions

There is a need for both *in vitro* and *in vivo* TdP proarrhythmia models. Results from these types of studies may help to increase knowledge of arrhythmogenic mechanisms (help to identify new parameters of proarrhythmia) as well as to substantially improve the predictive value of a pre-clinical battery of tests for proarrhythmic propensity of new drugs.

A multi-faceted testing strategy employing test systems of increasing complexity (addressing several parameters) is proposed: single cells, isolated tissue (wedge preparation), isolated heart, intact animal, and diseased (pathological) animal models. Parameters to be recorded include repolarization times (local and global), reverse frequency-dependence, spatial and temporal dispersion, EADs, and arrhythmias.

There is overall agreement that a multi-centre validation process is needed. The following recommendations are made:

(1) Testing laboratories should be qualified to perform these studies, for example, have a proven track record/ publications with the model;
(2) More than one site would be required to validate a particular model and studies should be allocated internationally;
(3) Studies should be conducted and analysed in a blinded fashion;
(4) Studies should be standardized across sites and be reproducible; and
(5) At least two species, preferably rabbit and dog, are needed for validation purposes.

Key considerations for demonstrating utility of pre-clinical models

Introduction

It appears that the current clinical ICH E14 QT guideline is having a significant effect on the discovery pipeline (ICH

HARMONIZED TRIPARTITE GUIDELINE E14 (2005); also see Bass *et al*., 2008 and Sager, 2008 in this special issue of the *British Journal of Pharmacology*). Owing to the concern that a drug will have a QT effect and that this will result in significant drug development challenges and regulatory hurdles, many companies are stopping the development of new molecular entities (NMEs) that have a pre-clinical signal suggesting QT liability (for example, hERG IC_{50}/free clinical plasma level ratio of less than 30- to 100-fold (Redfern *et al*., 2003) or in some cases even less than 200-fold).

Most pre-clinical assays currently used to screen NMEs for proarrhythmic liability focus on the 'QT liability', the potential of a drug to prolong the QT interval. It is, however, appreciated that important drugs with hERG activity have been developed (for example, verapamil, sodium pentobarbital) that do not have an arrhythmia risk. Although it would be optimum to lack any hERG activity, such an approach may hinder or halt the development of agents that are both clinically important and safe. Intense research is therefore ongoing with the focus of developing models that would allow drug developers to better distinguish drugs, which truly cause proarrhythmias (associated QT prolongation or with other mechanisms, for example, sodium channel blockade) from drugs, which prolong the QT interval but that are devoid of proarrhythmic liability. As new assays are developed and proposed to be used in the screening process, the important question on how to validate these assays emerges. The purpose of this discussion, therefore, is to reach consensus for future work on the following issues:

(1) Against which clinical end points should pre-clinical proarrhythmia assays be validated?
(2) How are clinical outcome data best captured?
(3) How should a validation programme of proarrhythmia assays be designed to provide compelling evidence of their predictive value?

Discussion

Challenges to selection of drugs, against which to validate proarrhythmia models. Proarrhythmias, such as TdP, may be life threatening or even fatal and it is therefore critical to have a low proportion of false negatives with pre-clinical proarrhythmia models, which translates into a high negative predictive value for the test. A high level of model sensitivity (that is, the proportion of drugs with proarrhythmic liability identified by the test) is also vital for the approach to be successful and for their acceptance by the industry, the medical community and regulators. Validation of any pre-clinical model, or battery of models, should be conducted against a relatively large number of drugs (which presumably will be driven by practical concerns rather than statistical approaches), for which the accumulated patient exposure must be substantial. 'Sufficiently' large patient exposure may be approached and defined using statistical methods, with predefined criteria for the lowest detectable incidence of TdP (such as 1 in 100 000 or one in one million patient months; Brass *et al*., 2006). It is acknowledged that this is a challenging task because of the low incidence of TdP with non-cardiovascular drugs (Wysowski *et al*., 2001; Barbey *et al*., 2002), and as the reporting rate may vary with the type of drugs and awareness among health professionals.

It is important to validate a pre-clinical strategy against drugs that are associated with TdP but have a relatively small QT effect, as these drugs may be more difficult to detect than ones with a large effect on cardiac ventricular repolarization. For specificity determinations, drugs that have small QT effects but no definitive evidence of clinical proarrhythmias (category b below) should also be examined. By validating the pre-clinical approach against drugs that affect multiple ion channels, the results can be more readily generalized. In addition, autonomic perturbations may also result in QTc prolongation (Cuomo *et al*., 1997; Frederiks *et al*., 2001; Piccirillo *et al*., 2001; Diedrich *et al*., 2002). For some drugs these QTc increases may not represent actual effects on ventricular repolarization, but instead result from an artificial increase in the QTc resulting from imperfect QT correction methodologies (such as overcorrection at high and undercorrection at low heart rates with the use of QTcB (Malik, 2001). Thus, it is important to include drugs in the validation that have autonomic effects and possibly also vasodilators, which increase the heart rate through baroreceptor-mediated mechanisms.

There is a consensus that the appropriate end point of pre-clinical proarrhythmia models should be the propensity of a drug to cause arrhythmias in man, with a focus on TdP, and not merely QT prolongation. Although TdP is the major clinical concern, the ability to exclude other forms of proarrhythmia should also be considered in developing a pre-clinical testing strategy. Thus, it will be necessary to validate the pre-clinical approach against hard end points such as clinical events. Greater confidence in the ability of pre-clinical models to reliably determine the risk of TdP and proarrhythmia, might reduce the attrition of NMEs during the discovery process while permitting the clinical development of safe medications.

Clearly the accurate definition of whether a drug is associated with proarrhythmia is critical and represents a challenge. Although there are already a number of drugs whose risk for causing TdP is well defined (cisapride, terfenadine, astemizole, droperidol, grepafloxacin, levomethadyl, lidoflazine, sertindole, terodiline) and robust data exist, it will be important to classify additional agents and to assess newly approved drugs prospectively for TdP risk. For example, there are several agents that prolong indices of ventricular repolarization, but have been suggested as not posing a proarrhythmic risk (for example, ranolazine and ziprasidone). The clinical experience of these recently marketed drugs is, however, not yet sufficiently large to allow any definitive conclusions in regard to proarrhythmic liability, given the extremely low background incidence of TdP in the general patient population. Furthermore, there are considerable limits to post-marketing reporting databases; TdP may not be accurately diagnosed, for example because of the lack of ECG data, patients may be taking other drugs associated with TdP, patients may be critically ill and patients might present with sudden death, obscuring a TdP diagnosis. Unexpected sudden cardiac death, particularly in young individuals without cardiovascular risk factors may be utilized as a surrogate for TdP if other possible mechanisms

(for example, thrombosis) can be reasonably excluded. One approach to determining the proarrhythmia risk is to use a blinded formal adjudication process performed by a group of arrhythmia experts to ascertain cases associated with an individual drug. Even so, when there are only a small number of isolated cases, it is difficult to be confident that a drug actually is proarrhythmic. An alternative approach may be to classify the drugs that will be used to validate the models, according to publicly available sources, such as the University of Arizona Health Sciences Center 'Drugs that Prolong the QT interval and/or induce Torsades de Pointes ventricular arrhythmia' (Anon, 2006). This approach recognizes that there is a large uncertainty regarding the quality of reports and that the denominator often is unknown. The report classifies drugs into the following categories (with potential further division into subcategories): (a) drugs with risk of TdP; (b) drugs with possible risk of TdP and (c) drugs unlikely to cause TdP. A similar approach has been used both for validation of pre-clinical tests (Redfern *et al*., 2003) and in an epidemiological study addressing sudden cardiac death (Straus *et al*., 2005). Other ways to study the proarrhythmic liability of individual drugs include epidemiologic studies, such as a recently published study on erythromycin, which demonstrated an increase in sudden death associated with co-administration of erythromycin and CYP3A inhibitors (Ray *et al*., 2004). There are, however, examples where similar approaches have failed to detect an increase in proarrhythmic events or sudden death, presumably because of small sample size or poor classification of clinical events in used databases (Hanrahan *et al*., 1995; Walker *et al*., 1999). Sufficient large study populations and improved event classification are important considerations in generating and evaluating these data.

Conclusions

It is proposed that a validation programme of a small battery of pre-clinical tests, which should include proarrhythmia assays, using the above principles can significantly move the field ahead. Such a validation programme would improve our understanding of the predictive value of these tests, and potentially help differentiate between drug-induced QT prolongation alone and risk of proarrhythmias.

Next steps

From the beginning, the goals of the HESI workshop, 'Moving Towards Better Predictors of Drug-Induced Torsade de Pointe (TdP)', were ambitious, 'to develop a better fundamental understanding of the emerging science, trends and methods and methodologies that relate to predicting drug-induced TdP'. From the discussions of each of the topic areas in this publication and the other publications in this special issue of the *British Journal of Pharmacology*, it is evident that in most cases identifying focused areas of research at this time is too limiting given the number of important questions that remain. Rather, each section of this publication provides several recommendations for further study to address many aspects of drug-induced torsades de pointes that remain to be considered. In other words, this paper provides a framework for structuring the major issues, identifies the state of knowledge, and describes the key areas of investigation, which hold the promise of leading to improved predictors of TdP. With this information, the scientific community is encouraged to consider the ideas advanced in this publication and to contribute to these important areas of investigations over the next several years.

Conflict of interest

The authors of this paper are employed in the pharmaceutical industry or serve as consultants to the pharmaceutical industry. However, the subjects presented in the paper do not advocate or support purchase of any of the products offered by the respective organizations.

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