Advantages of the Perfused Isolated Heart for the Assessment of the Electrophysiologic and Hemodynamic Effects of Drug Candidates

Over the past 20 years, several non-cardiac drugs have received intense scrutiny due to unanticipated reports of morbidity and mortality associated with adverse cardiac events, namely an increased incidence of life-threatening arrhythmias. More than 100 clinically used drugs, from a variety of unrelated classes, have been marked as possibly proarrhythmic (1, 2). Potential to trigger lethal arrhythmias has become a leading cause of removal of drugs from the market (1). The International Conference on Harmonization firmly advocates that preclinical and clinical tests for hemodynamic effects and proarrhythmic potential are performed for all new pharmaceutical compounds (3). Determination of a compound's cardiovascular side effects is paramount prior to a drug candidate receiving approval by government regulatory agencies, regardless of the intended therapeutic application.

Of particular interest in cardiovascular safety assessment are the electrophysiologic effects of an agent. The electrocardiographic QT interval currently serves as the primary surrogate indicator to gauge the proarrhythmic potential of drug candidates. However, other measures, such as changes in the duration of monophasic action potential (MAP), can also assist in evaluating the proarrhythmic potential of a compound. An electrocardiogram (ECG) reflects the propagation of action potentials waves Q, R, S and T) depicts ventricular depolarization and repolarization. It is controlled by the flux of sodium, calcium and potassium ions through their corresponding channels located on the cardiomyocyte membranes. The flow of potassium through the delayed rectifying potassium channel, namely the rapid component of this current, referred to as I_{Kr}, appears to be critical in repolarization and carries significant influence over changes in the QT interval. Torsades de Pointes, a potentially lethal ventricular tachyarrhythmia associated with QT interval prolongation, has become a primary concern in the study of drug-induced arrhythmia. Torsades de Pointes is characterized electrocardiographically by polymorphic QRS complexes oscillating around the isoelectric baseline.

Currently, no in vitro nor in vivo model can single-handedly predict the proarrhythmic capacity of a compound. In vitro testing assessing I_{Kr} channel inhibition represents one method by which therapeutic agents are initially screened for proarrhythmic potential. Though this may be an inexpensive and relatively straightforward technique, it does not account for other mechanisms that

CorDynamics

can cause arrhythmias, such as alterations in sodium channel function (4, 5). Basing the advancement of drug candidates solely on I_{Kr} channel inhibition will likely eliminate potentially beneficial therapeutic agents from further development. For instance, a variety of clinically important drugs block the I_{Kr} channel but have not been shown to trigger lethal arrhythmias (1, 6, 7, 8). This is likely due to additional counteractive mechanisms exerted by these drugs, such as modification of other cardiac ionic currents (6).

The electrophysiologic effects of novel drugs can also be assessed in isolated cardiac tissues, such as Purkinje fibers and papillary muscle. Though these assays offer a higher level of integration compared to isolated cell systems, they alone are not adequate since action potential profiles and distribution of ion channel subtypes differ throughout the myocardium. This may be important since heterogeneity of repolarization in the myocardium can contribute to arrhythmogenesis (9, 10). Ultimately, all drug candidates must be tested in whole animal models prior to clinical trial. While this type of examination offers the most complete preclinical assessment of a compound's proarrhythmic capabilities, it is not economically feasible, nor is it practical in regards to labor intensity or drug supply, to examine all promising candidates in vivo. Therefore, the data derived from in vitro assays must be as comprehensive as possible in order to select the most promising drug candidates for further testing.

The isolated heart model is a powerful system with many advantages over the above experimental preparations. However, its benefits have not been leveraged. This paradigm acts as a physiologically relevant bridge between purely in vitro assays and costly, resource-intensive whole animal studies. The model has been used extensively in experimental cardiovascular investigations and it is a widely accepted surrogate for the study of human cardiac function. Indeed, it has been demonstrated systematically that isolated rabbit hearts exhibit the same electrophysiologic responses as humans to numerous therapeutic compounds from a variety of classes (11, 12, 13). Generally, agents which have been implicated as proarrhythmic also prolong APD or QT interval in the isolated rabbit heart (11). In addition, rabbit hearts display similar responses to drugs with respect to hemodynamic effects compared with human myocardium. As noted in ICH S7A (3), unanticipated actions on myocardial pump function and/or the coronary vasculature should also be considered when determining the cardiac safety of drug candidates.

Recognizing the shortcomings of the aforementioned in vitro preparations, the isolated heart is an ideal model for determining the cardiovascular safety profile of promising candidates. As an example, these studies could be conducted on groups of favorable compounds within a project/program for lead optimization purposes. The model features greater throughput compared to complicated in vivo studies and provides a more comprehensive assessment of test article effects on cardiovascular function than currently utilized in vitro screens. Evaluation of a potential therapeutic agent's cardiovascular effects can be determined within a matter of days, including dose-response relationships and an adequate number of subjects to generate statistically meaningful data. The preparation does not require the

extensive amount of resources involved with in vivo models. These whole animal studies often require special drug formulations and pharmacokinetic analysis, issues that are rarely applicable to the isolated heart. In addition, the isolated heart requires considerably smaller quantities of drug compared to in vivo studies, a particularly attractive feature for compounds that are difficult to synthesize in the initial stages of development, and thus may only be available in limited amounts. It also allows for a range of concentrations and/or different combinations of compound(s) to be tested. This becomes especially important when examining situations where test article levels reach supratherapeutic levels, as in overdose or in patients with impaired drug metabolism (14-17).

Variables that contribute to the development of cardiac arrhythmias, such as heart rate and ionic concentration of the extracellular milieu, can be modified in the isolated heart model for evaluation of a drug's effects under various simulated clinical conditions. Control over the rate of pacing reduces fluctuations in ECG readings due to the dependence of QT interval on intrinsic heart rate, an important variable to be noted in vivo. Certain medications may only alter heart rate and falsely appear to modify QT interval, an effect easily unmasked in the isolated rabbit heart by controlled pacing. In addition, some drugs exhibit rate-dependent blockade of ion channels, such as Class I and Class III antiarrhythmics, which could affect dosing recommendations (18). Additionally, the ionic concentration of the perfusate can be modified to mimic some of the aberrant conditions that may be encountered in patients, such as hypokalemia, which can increase their arrhythmic sensitivity to QT interval prolonging drugs (19). Similar to rate dependent blockade, certain drugs, such as quinidine and dofetilide, also exhibit differential ion channel binding dependent on extracellular ion concentration (20, 21).

In summary, the isolated heart possesses numerous advantages as a cost-effective and informative screen for the cardiovascular safety assessment of new drug candidates. This model possesses a higher level of integration than isolated cell and tissue assays, yet is considerably less resource-intensive compared to in vivo models. Removing potentially hazardous compounds early in the drug development scheme facilitates the selection of more promising drug candidates, as well as decreases expenditures on the more complicated assays used in the later stages of preclinical development. The knowledge gained by examining compounds in this model significantly improves the chance of bringing promising candidates forward more rapidly, safely, and cost-effectively.

Advantages of the Isolated Heart Model

• Higher level of integration compared to isolated cell and tissue assays

• Greater throughput compared to in vivo models

• Multi-dimensional assessment of a compound's cardiovascular effects

• Ability to perform dose response relationship experiments and generate statistically meaningful data
  in 1-2 days, per compound studied

• Considerably less labor, cost and time compared to in vivo models

• High level of reproducibility

• Requires small amounts of test compound

• Allows for a range concentrations and/or different combinations of medication(s) to be tested

• Can be manipulated to mimic clinical conditions (hypokalemia, bradycardia, etc.)

Parameters That Can be Assessed in the Isolated Heart Model

Electrophysiologic

> • Heart rate
>
> • Monophasic Action Potential Duration
>> • Sodium Channel Parameters
>> • Calcium Channel Parameters
>> • Potassium Channel Parameters
>
> • PR interval
>
> • QRS duration
>
> • QT interval
>
> • Arrhythmias
>
> • Cycle length dependence

Hemodynamic

> • Ventricular Pressure parameters
>> • Left ventricular end diastolic pressure
>> • Left ventricular systolic pressure
>> • Left ventricular developed pressure
>
> • Coronary perfusion pressure (vascular resistance)
>
> • Contractility assessment
>> • +dP/dt (inotropy)
>> • -dP/dt (lusitropy)

References

1.   Fermini B, Fossa AA. The impact of drug-induced QT interval prolongation on drug discovery and development. Nature Drug Discovery. 2003;2:439-447.

2.   Haverkamp W, Breithardt G, Camm AJ, Janse MJ, Rosen MR, Antzelevitch C, Escande D, Franz M, Malik M, Moss A, Shah R. The potential for QT prolongation and pro-arrhythmia by non anti-arrhythmic drugs: clinical and regulatory implications report on a policy conference of the European Society of Cardiology. Cardiovasc Res. 2000;47: 219-233.

3.   International Conference on Harmonisation. S7A Safety Pharmacology Studies for Human Pharmaceuticals (2001) and S7B Safety Pharmacology Studies for Assessing the Potential for Delayed Ventricular Repolarization (QT interval prolongation) By Human Pharmaceuticals (2003).

4.   Wang Q, Shen J, Li Z, Timothy K, Vincent GM, Priori SG, Schwartz PJ, Keating MT. Cardiac sodium channel mutations in patients with long QT syndrome, an inherited cardiac arrhythmia. Human Mol Genet. 1995;4:1603-1607.

5.   Wang Q, Shen J, Splawski I, Atkinson D, Li Z, Robinson JL, Moss AJ, Towbin JA, Keating MT. SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. Cell. 1995;80:805-811.

6.   Cavero I, Mestre M, Guilon J-M, Crumb W. Drugs that prolong QT interval as an unwanted effect: assessing their likelihood of inducing hazardous cardiac dysrhythmias. Exp Opin Pharmacother. 2000;1:847-973.

7.   Zhang S, Zhou Z, Gong Q, Makielski JC, January CT. Mechanism of block and identification of the verapamil binding domain to HERG potassium channels. Circ Res. 1999;84:989-998.

8.   Thomas D, Gut B, Wendt-Nordahl G, Kiehn J. The antidepressant drug fluoxetine is an inhibitor of human ether-a-go-go-related gene (HERG) potassium channels. J Pharmacol Exp Ther. 2002;300:543-548.

9.   Zareba W, Badilini F, Moss AJ. Automatic detection of spatial and dynamic heterogeneity of repolarization. J Electrocardiol. 1994;27:66-72.

10.   Vaughn Williams EM. Relevance of cellular to clinical electrophysiology in interpreting antiarrhythmic drug action. Am J Cardiol. 1989;64:5J-9J.

11.   Hondeghem LM, Carlsson L, Duker G. Instability and triangulation of the action potential predict serious proarrhythmia, but action potential duration prolongation is antiarrhythmic. Circulation. 2001;103:2004-2013.

12.   Hondeghem LM, Hoffman P. Blinded test in isolated female rabbit heart reliably identifies action potential duration and prolongation and proarrhythmic drugs: importance of triangulation, reverse use dependence and instability. J Cardiovasc Pharmacol. 2003;41:14-24.

13.   Hondeghem LM, Lu HR, van Rossem K, De Clerck F. Detection of proarrhythmia in the female rabbit heart: blinded variation. J Cardiovasc Electrophysiol. 2003;14:287-294.

14.   Owens RC Jr. Risk assessment for antimicrobial agent-induced QTc interval prolongation and torsades de pointes. Pharmacotherapy. 2001;21:301-319.

15.   Pohjola-Sintonen S, Viitasalo M, Toivonen L, Neuvonen P. Itraconazole prevents terfenadine metabolism and increases risk of torsades de pointes ventricular tachycardia. Eur J Pharmacol. 1993;45:191-193.

16. Goldschmidt N, Azaz-Livshits T, Gotsman I, Nir-Paz R, Ben-Yehuda A, Muszkat M. Compound cardiac toxicity of oral erythromycin and verapamil. Ann Pharmacother. 2001;35:1396-1399.

17. van Haarst AD, van't Klooster GA, van Gerven JM, Schoemaker RC, van Oene JC, Bruggraaf J, Coene MC, Cohen AF. The influence of cisapride and clarithromycin on QT intervals in healthy volunteers. Clin Pharmacol Ther. 1998;64:542-546.

18. Weirich J, Antoni H. Rate-dependence of antiarrhythmic and proarrhythmic properties of class I and class III antiarrhythmic drugs. Basic Res Cardiol. 1998;93:125-132.

19. Roden DM, Woosley RL, Primm RK. Incidence and clinical features of the quinidine-associated long QT syndrome: implications for patient care. Am Heart J. 1986;111:1088-1093.

20. Roden DM, Hoffman BF. Action potential prolongation and induction of abnormal automaticity by low quinidine concentrations in canine Purkinje fibers. Relationship to potassium and cycle length. Circ Res. 1985;56:857-867.

21. Yang T, Roden DM. Extracellular potassium modulation of drug block I_{Kr}. Implications for torsades de pointes and reverse use-dependence. Circulation. 1996;93:407-411.