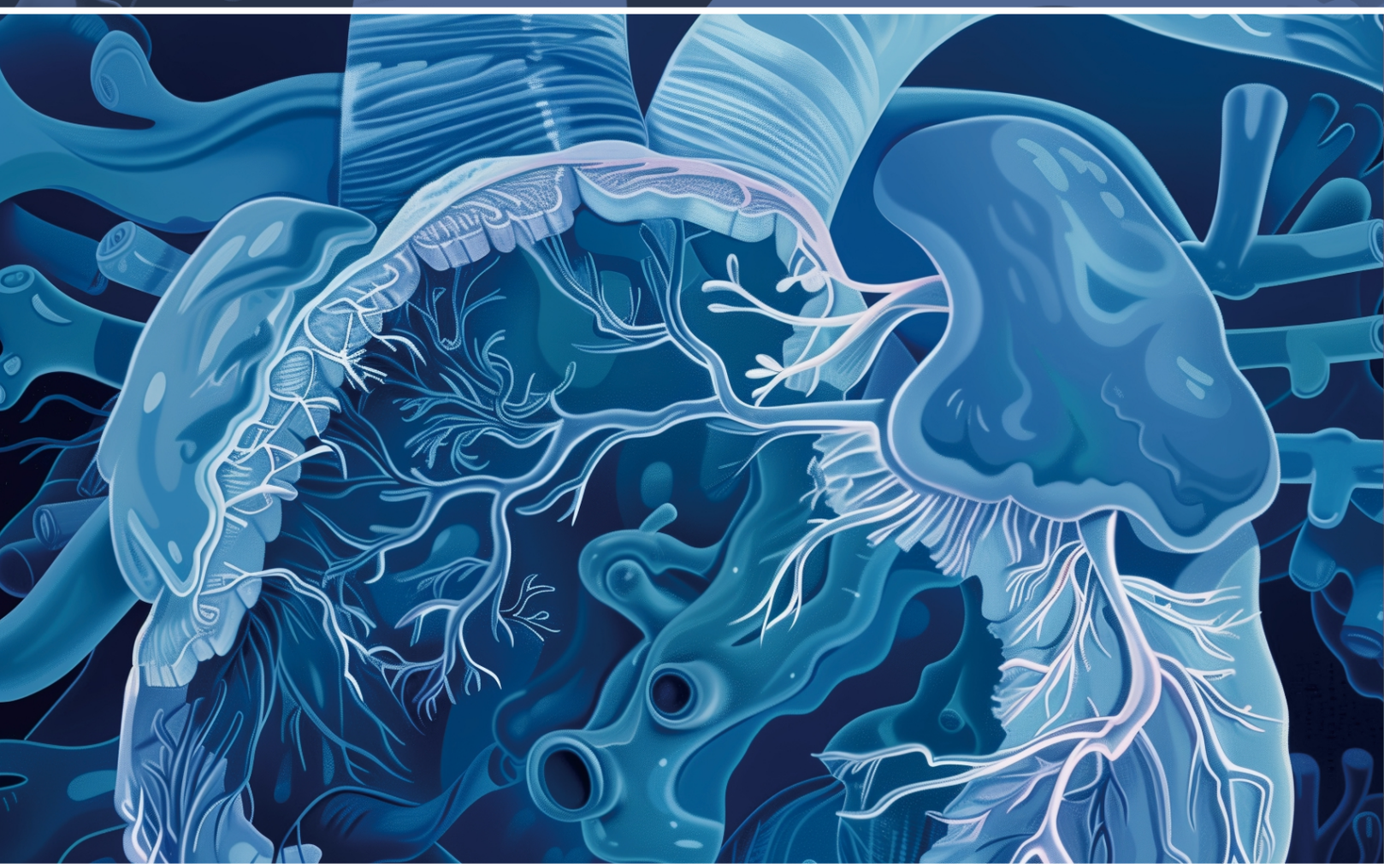
Cardiac Safety Assessment

Strategies for Comprehensive Cardiac Profiling



Innovative CRO+Explorer
ICE Bioscience

Cardiac safety assessment is a critical component of the drug development process, aimed at identifying potential adverse effects of pharmaceutical compounds on the heart. The significance of these evaluations lies in their capacity to prevent drug-induced cardiac arrhythmias, which can lead to serious health outcomes, including sudden cardiac death. By systematically identifying and mitigating these risks early in drug development, cardiac safety assessments protect patient health and contribute to the development of safer therapeutic options.

The landscape of cardiac safety assessment is critically informed by the ICH S7B guideline, an essential framework established to guide the non-clinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by pharmaceuticals. This prolongation can increase the risk of cardiac arrhythmia, notably Torsades de Pointes (TdP), a potentially life-threatening condition. As part of a comprehensive safety evaluation, S7B offers a foundational approach for in vitro and in vivo studies to assess the risk of drugs to cause such adverse effects.

In 2022, the regulatory community welcomed the much-anticipated update to the S7B guideline, accompanied by a detailed Q&A document. This update represents a significant advancement in the field, incorporating the latest scientific understanding and methodologies. The 2022 S7B Q&A clarifies best practices and provides additional guidance on the implementation of these non-clinical evaluations. Key highlights include the emphasis on integrated risk assessments, leveraging both in vitro and in vivo data to predict QT interval prolongation more accurately.

Integrated Evaluation Strategy at ICE Bioscience

Hit to Lead	Lead Optimization	Candidate Selection
Tier-1 Risk Assessment	**Tier-2 Risk Assessment**	**Tier-3 Risk Assessment**
hERG screening: single dose or dose-response analysis Manual or automated patch-clamp technology	hERG screening: single dose or dose-response analysis Manual or automated patch-clamp technology	Comprehensive hERG assays to establish the dose-response relationship (IC50) in accordance with best practices
Evaluation of additional key cardiac ion channels, Ca_v1.2 and Na_v1.5, for single dose analysis	Evaluation of additional key cardiac ion channels, Ca_v1.2 and Na_v1.5, for single dose or dose-response (IC50) analysis	Evaluation of additional key cardiac ion channels, Ca_v1.2 and Na_v1.5, for complete dose-response (IC50) analysis
Optional QTc interval assessment in a guinea pig model for in vivo cardiac electrophysiology analysis	Action potential assays in induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) (Optional)	Action potential assay in iPSC-CMs or rabbit Purkinje fiber
		Optional QTc interval assessment in a guinea pig model for in vivo cardiac electrophysiology analysis
√ Early hazard identification and off-target mitigation √ Ensure the safety profile of the lead compound √ Achieving an optimal balance between efficacy and efficiency	√ Hazard Elimination √ Facilitates Structure-activity Relationship (SAR) Model Development for Compound Optimization √ Mitigation of Potential Liabilities Informed by SAR Analysis	√ Mechanism of Action Elucidation √ IND Support: Essential documentation and regulatory assistance for IND applications. √ Decision-Making Support: Strategic guidance based on robust data analysis.

Best Practice Considerations for In vitro Studies: hERG assays

ION CHANNEL RECORDING

Measuring ion channels:

- Recording temperature
- Voltage protocol
- Recording quality

- Recording temperature: Maintain a temperature close to physiological levels (35-37°C) to simulate clinical conditions accurately.
- Voltage protocol: Mimic ventricular action potential waveform and repeat stimulation at physiological frequency.
- Recording quality: Ensure adequate voltage control and stable cell and recording properties.

DATA ANALYSIS APPROACH

Analyzing data:

- Primary endpoint measures
- Data summary

- Reporting parameters: Provide values for the half inhibitory concentration (IC50), Hill coefficient, and uncertainty estimation (95% confidence interval or CI).
- Justification for highest concentration: If 50% current inhibition is not achieved, a justification of the highest concentration tested should be provided.
- Data summaries: Include summaries of inhibition rate, IC50, input resistance, and current time course.

COMPOUND HANDLING

Handling the compound:

- Concentration verification
- Positive and negative controls

- The concentration of test compound to which the cells were exposed should be verified by applying a validated analytical method to the solution collected from the cell chamber.
- The positive control drug should be tested using sufficient replicates and two or more concentrations achieving 20-80% block.

hERG Assays at Room Temperature - Using MPC and APC

- Manual patch-clamp is the gold standard for ion channel research, allowing for precise control over experimental conditions, and offering precise and detailed screening data. However, it is technically demanding and unsuitable for high-throughput screening.
- The automated patch-clamp technique enables higher throughput screening compared to MPC, allowing for faster data collection and analysis. APC reduces the need for manual labor, leading to cost savings in screening processes. However, the automated nature of the process may result in slightly decreased data precision compared to manual patch-clamp. APC may also have limitations in terms of the range of experimental conditions that can be controlled compared to manual patch-clamp setups.

ICE Bioscience offers both MPC and APC services, providing flexibility to meet the diverse needs of our clients. Whether you require the precision and detail of manual patch-clamp or the higher throughput of automated patch-clamp, we have the solutions to accommodate your research requirements.

Accomplishments of hERG Projects in 2023

3500+
Compounds Tested for hERG Assays

1500+
hERG projects totally

38
projects supporting IND filings globally

Drugs	Manual Patch Clamp IC50	Automated Patch Clamp IC50
Cisapride	14.4nM	28.78nM
Amitryptyline	3700nM	5732.26nM
E4031	11.0nM	24.32nM
Quinidine	596.6nM	1124.97nM
Mexiletine	55900nM	50174.71nM
Verapamil	169.6nM	636.67nM
Ranolazine	8000nM	8811.64nM
Nifedipine	170500nM	133655.92nM

Table 1: Comparison of Compounds Identified by Manual and Automated Patch-Clamp Systems

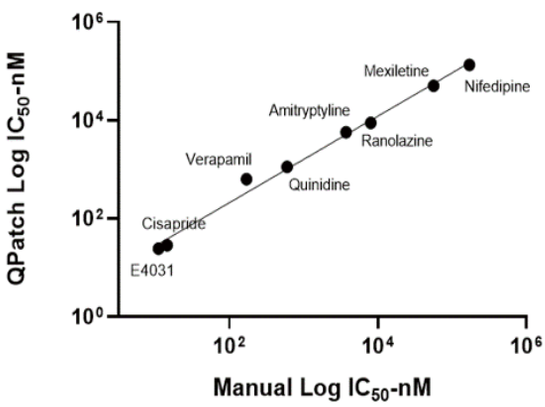


Figure 1. Correlation between the hERG IC50 values obtained using manual patch clamp and those obtained using automatic patch clamp.

hERG Assays at Physiological Temperature

Adhering to the latest ICH E14/S7B Q&A guidelines, our service now offers advanced in vitro IKr/hERG assays. We ensure recording at physiological temperatures (35-37°C), apply voltage protocols mirroring the ventricular action potential, and use analytical methods for accurate compound concentration validation. Our assays feature robust controls for precise detection, reflecting a commitment to bridging non-clinical findings with clinical research.

- Recording temperature should close to physiological temperature (35-37°C) to better predict the compound's effects on ion channels under clinical conditions;
- Voltage protocols that approximate the ventricular action potential waveform, with repetitive stimulation at physiological frequencies;
- Appropriate clamping voltage, stable cell and recording properties;
- Concentrations of compounds acting on cells are validated by concentration-validated analytical methods on solutions collected from cell chambers;
- Positive control drugs should have sufficient replicates and two or more concentrations reaching 20-80% blockade for detection.

	Room Temperature（IC50）	Physiological Temperature（IC50）
E-4031	20.38 nM	10.58 nM
Terfenadine	16.64 nM	27.69 nM
Cisapride	16.37 nM	18.16 nM

Table2. Comparison of hERG IC50 values under physiological and room temperature conditions. E4031 and terfenadine exhibit different inhibitory effects on hERG channels at physiological temperature and room temperature. Cisapride exhibits similar inhibitory effects on hERG channels at physiological temperature and room temperature.

Nav/Cav Assays at Room and Physiological Temperature

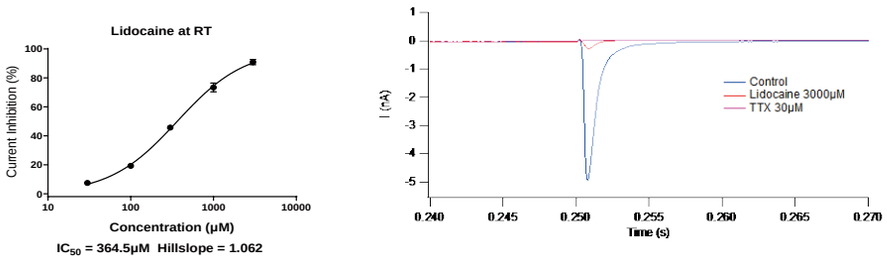


Figure 5. Concentration-response curves and current recordings for the effects of Lidocaine on Nav1.5 channels under room temperature conditions.

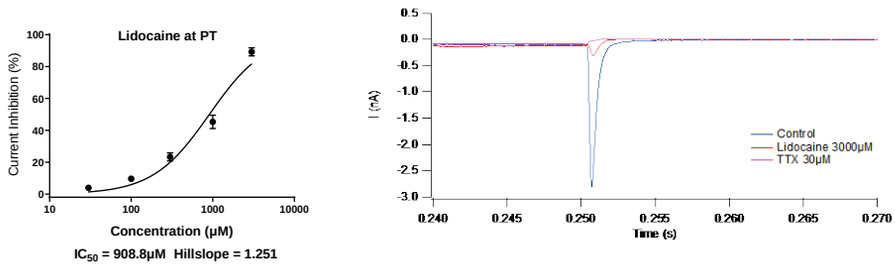


Figure 6. Concentration-response curves and current recordings for the effects of Lidocaine on Nav1.5 channels under physiological temperature conditions.

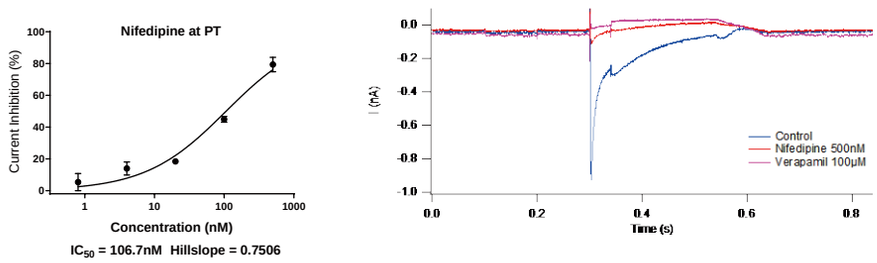


Figure 7. Concentration-response curves and current recordings for the effects of Nifedipine on Cav1.2 channels under physiological temperature conditions.

CiPA Ion Channel Panel Screening

Our CiPA Ion Channel Panel Screening service meticulously focuses on seven critical cardiac ion channels: hERG, Cav1.2, Nav1.5, Nav1.5-Late, IKs, IK1, and Kv4.3. These channels were selected for their established roles in cardiac repolarization and rhythm regulation, factors crucial to evaluating proarrhythmic risk. The manual patch clamp method allows for precise measurements, providing detailed insights into each channel's pharmacological response, which is vital for comprehensive cardiac safety assessments.

Target	Description
hERG	An assessment of drug effects on hERG channels, related to the prolongation of the QT interval on the electrocardiogram (ECG)
Cav1.2	Investigation of drug effects on Cav1.2 channels, which are associated with the duration of the action potential (APD) and could potentially affect hERG-related QT prolongation
Nav1.5	Analysis of drug interactions with Nav1.5 channels that are linked to potential risk for arrhythmias and cardiac adverse events
Nav1.5-Late	Study of late sodium currents (INaL) and their potential association with early afterdepolarizations (EADs), which can be a risk factor for arrhythmias
IKs	Examination of drug effects on IKs channels, also related to QT interval modulation
IK1	Assessment of drug impact on IK1 channels, relevant for maintaining the resting membrane potential of cardiac cells
Kv4.3	Analysis of drug effects on Kv4.3 channels, which play a role in cardiac repolarization

Action Potential Assessment

Using iPSC Derived Cardiomyocytes

The CiPA initiative recognizes the critical role of induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) in evaluating the proarrhythmic potential of new drugs. This approach leverages iPSC-CMs to study the integrated response of human cardiac action potentials to pharmaceutical compounds, focusing on the collective behavior of multiple cardiac ion channels. APD90 represents the duration it takes for the cardiac action potential to repolarize to 90% of its amplitude. It's a critical parameter for assessing cardiac safety, as drugs affecting APD90 by prolonging it may increase the risk of arrhythmias like Torsades de Pointes, especially if the prolongation exceeds a certain threshold, commonly considered to be around 10-15%.

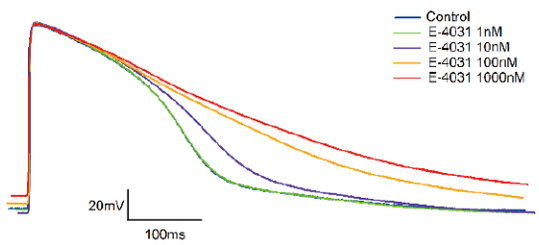


Figure 8. Effects of different concentrations of E-4031 on action potential of iPSC-CMs.

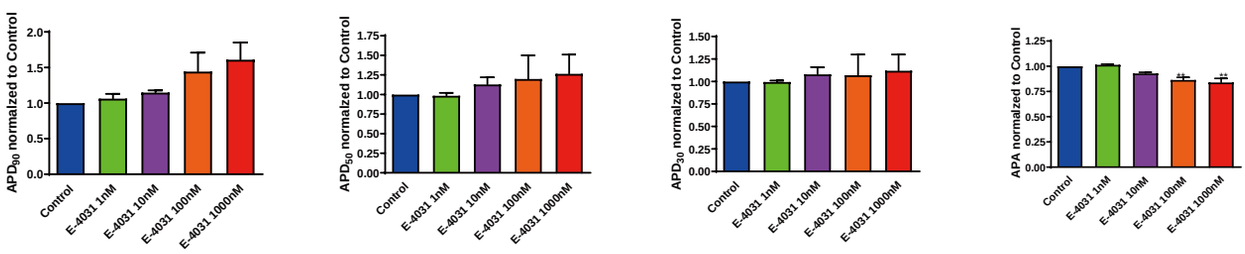


Figure 9. Effects of different concentrations of E-4031 on action potential duration at 90% repolarization (APD90), action potential duration at 50% repolarization (APD50), action potential duration at 30% repolarization (APD30), and action potential amplitude (APA) of iPSC-CMs.

Using Rabbit Purkinje Fiber

Rabbit Purkinje fibers, due to their physiological characteristics and electrophysiological properties, offer a unique advantage for detailed action potential studies, closely mimicking human cardiac tissue responses. They provide a more mature cardiac electrophysiology profile without the need for extensive differentiation protocols. This sophisticated in vitro technique is essential for predicting drug-induced QT interval prolongation and the risk of torsades de pointes. Leveraging the isolated rabbit Purkinje fibers, we meticulously analyze the drug effects on action potentials, providing a nuanced understanding of potential proarrhythmic liabilities.

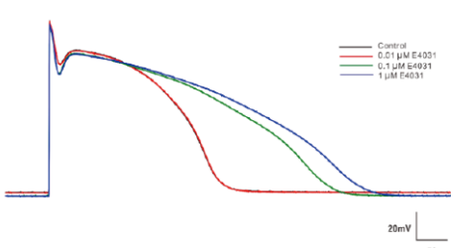
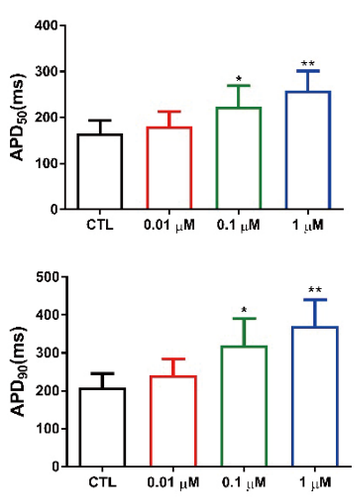


Figure 10. 0.1μM and 1μM E4031 significantly prolonged APD50 and APD90. Typically, a prolongation of 15% is considered the risk threshold.



Langendorff Perfusion Technique

Isolated mammalian hearts perfused in Langendorff mode have been utilized for over a century in cardiac physiology, pharmacology, toxicology, and pathology. This method features superfusion of the heart retrogradely via the aorta and measures left ventricular contraction with an inflated balloon placed in the ventricular cavity. It has gained increasing acceptance for elucidating mechanisms of arrhythmias and identifying the arrhythmogenic propensity of drugs. Using this model, hemodynamic functions and cardiac electrophysiology of interest, such as the QT interval and monophasic action potential duration, can be analyzed simultaneously. Multiple proarrhythmic variables closely associated with drug-induced Torsades de Pointes (TdP), such as dispersion of repolarization, triangulation, reverse-use dependence, and even overt arrhythmic events, can be readily analyzed in isolated Langendorff hearts.

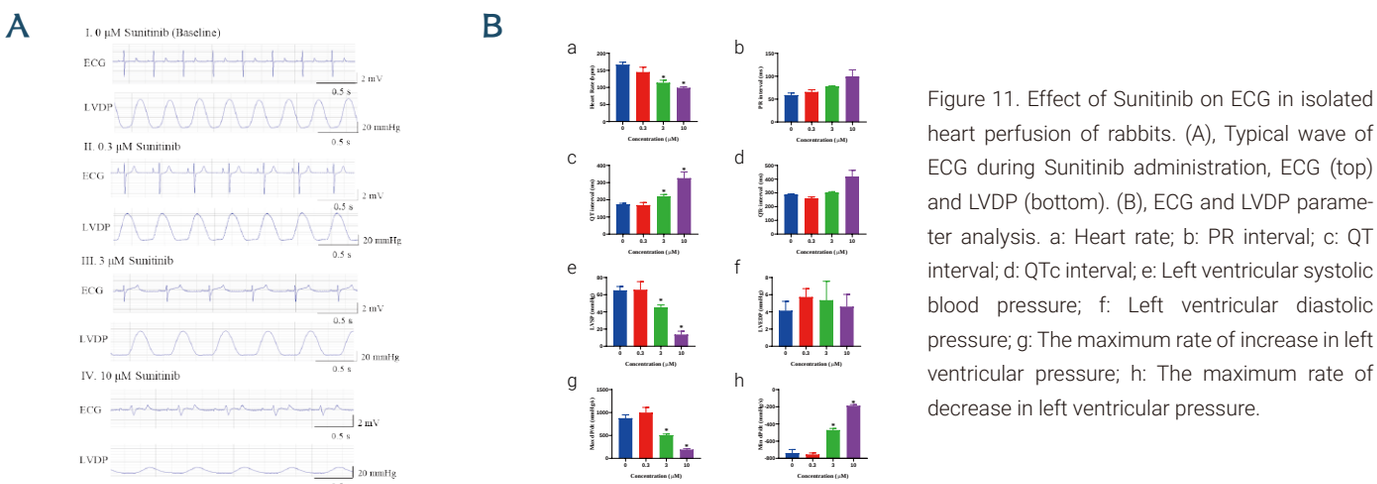


Figure 11. Effect of Sunitinib on ECG in isolated heart perfusion of rabbits. (A), Typical wave of ECG during Sunitinib administration, ECG (top) and LVDP (bottom). (B), ECG and LVDP parameter analysis. a: Heart rate; b: PR interval; c: QT interval; d: QTc interval; e: Left ventricular systolic blood pressure; f: Left ventricular diastolic pressure; g: The maximum rate of increase in left ventricular pressure; h: The maximum rate of decrease in left ventricular pressure.

Comparative Analysis of Guinea Pig Models in Cardiac Safety Evaluation

Cardiac proarrhythmic risk evaluation is an important segment that restricts the preclinical progress of various new drug research and development. QT interval of the electrocardiogram is indicative of ventricular AP repolarization delay. In experimental animals, the heart structure of large animals is closer to that of humans, making them have unique application values in predicting heart safety. However, their purchase and feeding costs are high, and they require large amounts of medication, while small animals can avoid these problems.

Research has found that the overall structure and function, action potential, ion channels, and electrocardiogram of small animal hearts are similar to those of humans in the order of rabbit>guinea pig>rat ≈ mouse. The contractility and calcium processing of rabbit and guinea pig hearts are more dependent on the characteristics of external calcium compared to rats and mice. Rabbits and guinea pigs have better sensitivity to cardiac glycosides, and guinea pig animal models are very suitable for detecting drug-induced changes in cardiovascular indicators. Guinea pigs are excellent models to investigate electrophysiologic and cardiovascular parameters. They are inexpensive and possess specific ion channels (with the exception of Ito) similar to those in humans.

Figure 12. Typical surface electrocardiogram (ECG) in guinea pig. (A) ECG in control group from healthy guinea pig. (B) ECG in model group of drug treated guinea pig. Heart rate was slowed, and QT interval was prolonged in the presence of drug.

ICE Bioscience
Innovative CRO+ Explorer

ICE Bioscience was founded in 2010 as an Innovative CRO+ Explorer company. We specialize in early drug discovery services, spanning from target validation to the identification of pre-clinical candidates. We stand out for our collaborative spirit and expertise in boldly exploring new therapeutic target research. Our commitment to drug discovery services, delivered with enthusiasm and professionalism, empowers clients to overcome challenges, address scientific puzzles, and fulfill our promises to clients, communities, the environment, and global health.

To learn more, please visit: en.ice-biosci.com