A comprehensive assessment of in vitro heart safety for antitumor drugs

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Introduction

The major safety concerns that lead to the termination of clinical drug development programs and the withdrawal of approved drugs from the market are related to the cardiovascular system and the liver. The most frequent cardiovascular adverse event that prompts drug withdrawals is the potentially life-threatening cardiac arrhythmia known as Torsades de Pointes (TdP). Sunitinib, a multi-targeted receptor tyrosine kinase inhibitor,has shown promise in treating various tumors but raises concerns regarding its cardiovascular safety. This study aims to comprehensively evaluate the proarrhythmic risks associated with Sunitinib, employing an integrated platform that spans both in vitro and ex vivo analyses.

Current cardiac safety testing guidelines are based on evaluating in vitro human ether-à-go-go-related gene (hERG) assays and in vivo electrocardiogram (ECG) assays as surrogates for human cardiac dysrhythmia. Although the inhibition of hERG channels is widely recognized and used as a sensitive indicator of drug cardiotoxicity, the hERG assay has significant limitations in predicting arrhythmias. Relying solely on hERG blockade to abandon promising compounds may lead to the premature termination of clinical trials for drugs that are actually safe. The Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative proposes an earlier evaluation of proarrhythmic risk based on a mechanistic understanding of the effects of candidates on multiple ion channel currents, moving away from the evaluation of QT interval changes as a surrogate for TdP risk. CiPA screening expands the panel of other cardiac ion channels, all of which play a key role in controlling the ventricular action potential (AP).

To further assess the drug's impact at a cellular level, we measure action potential dynamics in human iPSC-derived cardiomyocytes. This approach allows us to observe Sunitinib's effects on cellular excitability and rhythm. We also employ the Langendorff perfusion system to analyze Sunitinib's effects on isolated rabbit hearts. This ex vivo method provides insights into the drug's overall impact on cardiac function, including parameters such as ECG patterns and Left Ventricular Developed Pressure (LVDP). These comprehensive assessments aim to bridge the gap between cellular-level electrophysiology and whole-heart responses, offering a multi-dimensional view of Sunitinib's cardiac safety profile.

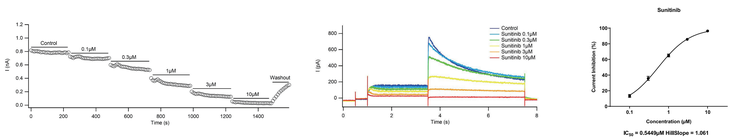
Materials and Methods

- In Vitro Cardiac Ion Channel Assays: Utilized CHO or HEK293 cells stably expressing hERG, Nav1.5, Cav1.2, and IKs channels, specifically developed in our laboratory. Employed patch clamp technique for precise measurement of ion channel activity and drug interaction.
- In Vitro iPSC-CMs Action Potential and CellTiter-Glo Assay: Applied Human iPSC-derived cardiomyocytes, sourced from a reliable supplier, for evaluating action potential alterations. Conducted CellTiter-Glo (CTG) assays for assessing cellular viability post-drug exposure.
- Ex Vivo Langendorff Perfusion Assay: Implemented this technique using rabbit hearts to examine the cardiac effects of drugs in a more integrated organ-level system.
  Data Analysis: Performed IC50 calculations and curve-fitting using GraphPad Prism software, ensuring accurate and reliable statistical analysis.

Results

1、hERG Channel Inhibition Assessment: Step and Ramp Protocols in Vitro

a. Step protocol



b. CiPA ramp protocol

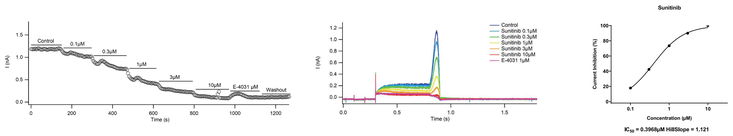
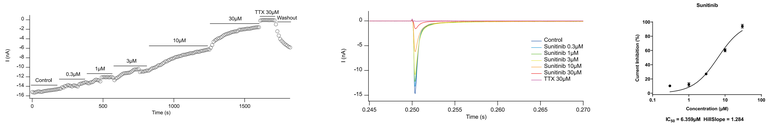


Figure 1: Assessment of in vitro hERG inhibition by Sunitinb using multiple voltage protocols with manual patch clamp.

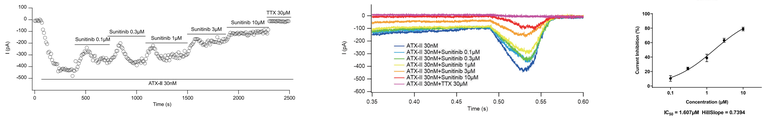
(a). Step protocol; (b). CiPA ramp protocol.

2、Comprehensive In Vitro Evaluation of Drug Effects on Multiple Ion Channels

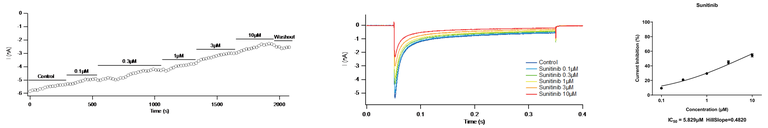
a. Nav1.5



b. Late-Nav1.5



c. Cav1.2



d. IKs

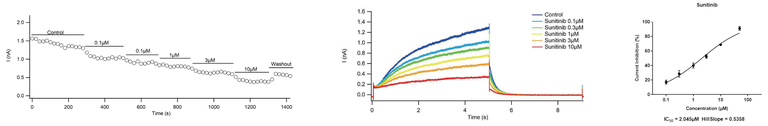


Figure 2: Assessment of in vitro multiple ion channels inhibition by Sunitinb using manual patch clamp.

(a). The effect of sunitinb on Nav1.5 channel including I-t plot graph, current trace, IC50 data.
(b). The effect of sunitinb on Late-Nav1.5 channel including I-t plot graph, current trace, IC50 data.
(c). The effect of sunitinb on Cav1.2 channel including I-t plot graph, current trace, IC50 data.
(d). The effect of sunitinb on IKs channel including I-t plot graph, current trace, IC50 data.

3、Comprehensive Analysis of iPSC-Derived Cardiomyocytes: Assessing Action Potential Dynamics and Cell Viability

Effects of Sunitinib on Action Potential of IPSC-CMs						
Concentration	APD30	APD50	APD90	APA	Vmax	RMP
(μM)	(Δ%)	(Δ%)	(Δ%)	(ΔmV)	(Δ%)	(ΔmV)
0.1	-0.14 ± 1.71	1.01 ± 1.95	2.16 ± 0.83	-2.12 ± 0.57	-7.61 ± 5.90	-2.58 ± 1.32
0.3	2.84 ± 3.63	5.54 ± 4.58	9.39 ± 4.15	-3.00 ± 0.70	-15.35 ± 5.93	-4.82 ± 2.55
1	8.40 ± 2.15	15.81 ± 3.34	36.99 ± 11.99	-6.57 ± 1.09	-34.53 ± 0.82	-8.22 ± 1.98
3	13.16 ± 4.71	27.60 ± 7.10	63.00 ± 19.84	-15.66 ± 2.11	-53.02 ± 4.63	-15.28 ± 0.15

Table 1: Effects of Sunitinib on action potential of iPSC-CMs. APD30 , APD50 and APD90, action potential duration measured at 30%, 50% and 90% repolarization; %, Percent change from baseline values (Mean SEM, n = 4); mV, absolute change from baseline in millivolts; RMP, resting membrane potential; APA, action potential amplitude; Vmax, maximum rate of depolarization.(a). Step protocol; (b). CiPA ramp protocol.

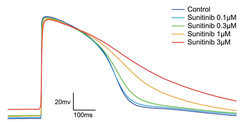


Figure 3: Detailed representative trace of action potential dynamics in iPSC-CMs following exposure to various concentrations of Sunitinib.

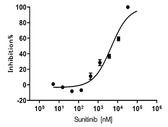


Figure 4: Cell viability was measured by CTG assay. CellTiter-Glo dye is used to determine the number of cells in cell culture by generation of a luminescent signal.

4、Langendorff Perfusion Technique: Assessing Sunitinib's Impact on Cardiac Function in Isolated Adult Rabbit Hearts

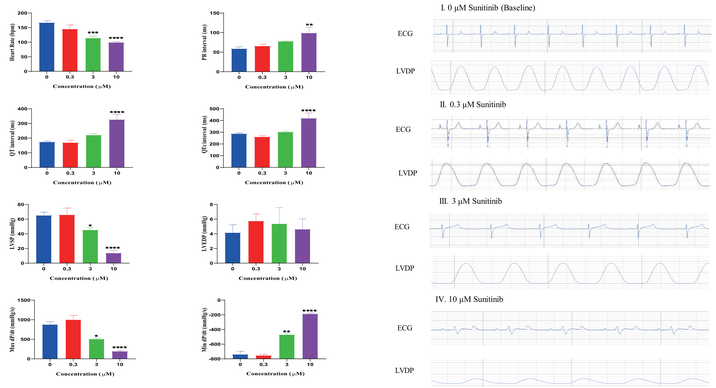


Figure 5: Effects of Sunitinib on isolated rabbit hearts. The study observed notable changes in ECG patterns and Left Ventricular Developed Pressure (LVDP) across these concentrations, indicating Sunitinib's influence on heart rhythm and contractility, highlighting a significant impact on cardiac performance, particularly at higher concentrations of Sunitinib.

Summary

This study provides a thorough assessment of Sunitinib's cardiac proarrhythmic risks using in vitro and ex vivo methods. In vitro assays revealed Sunitinib's inhibition on multiple ion channels including hERG, Nav1.5, Cav1.2, and IKs. iPSC-derived cardiomyocytes showed significant action potential prolongation at increasing Sunitinib concentrations. Ex vivo analysis using the Langendorff system on rabbit hearts indicated dose-dependent effects on heart rhythm and contractility. Collectively, these results underscore the importance of a comprehensive and integrated approach to cardiac safety assessment, revealing significant insights into the proarrhythmic potential of Sunitinib and emphasizing the necessity of careful dose management to mitigate cardiac risks.

References

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