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Ibrutinib induces cardiac arrhythmia by targeting $Ca_V1.2$ calcium channel

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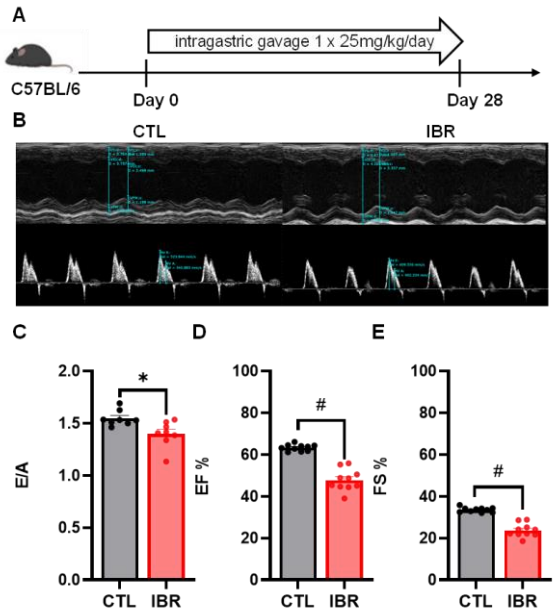
Introduction

Ibrutinib is a Bruton tyrosine kinase inhibitor that has shown significant efficacy against chronic lymphocytic leukemia (CLL). Clinical studies have found that ibrutinib increases the risk of cardiac arrhythmia, but the underlying molecular mechanisms are still unclear. $Ca_V1.2$ calcium channel is indispensable for the cardiac excitations and mediates calcium-induced calcium release, which maintain the intracellular calcium homeostasis. However, the functions of $Ca_V1.2$ channels are dysregulated, which indeed leads to cardiac arrhythmia. Here, we identified that Ibrutinib could regulate the $Ca_V1.2$ channel function, which might mediate its cardiac toxicity.

Results

1. Ibrutinib treatment resulted in impaired diastolic function

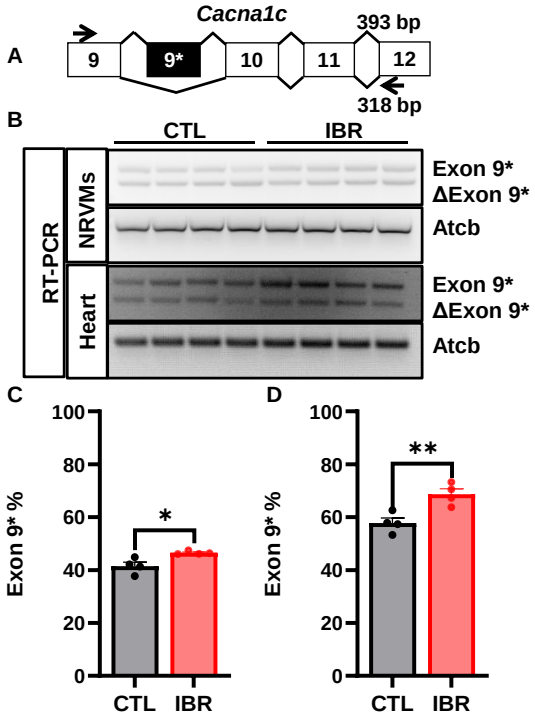


(A) The animal model was established by intragastric administration of ibrutinib 25mg/kg/day. (B) Cardiac function in untreated and post-treatment C57BL/6 mice was examined by echocardiography. Representative M-mode echocardiographic images of normal or post-treatment hearts. We found that after ibrutinib treatment, the E/A of the mouse heart was decreased (C), and the left ventricular ejection fraction (D) and shortened fraction were significantly different(E). **P*<0.05, #*P*< 0.0001, unpaired t-test.

Materials and Methods

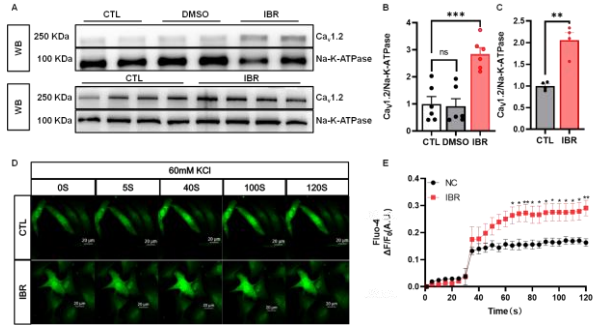
- Primary myocardium of neonatal rats isolation and culture
- Immunofluorescence staining
- ECG
- RT-PCR
- Western blotting
- Whole-cell patch clamp

2. Ibrutinib induces aberrant splicing of $Ca_V1.2$ channels in the heart



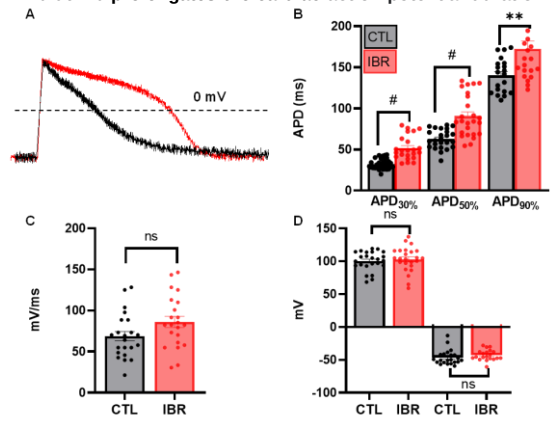
(A) Schematic illustration of PCR primers for amplification and detection of $Ca_V1.2$ with or without exon 9* in NRVMs and mouse heart tissue. (B) Total RNA was extracted from NRVMs and mouse hearts, and PCR products amplified from cardiac cDNA libraries were isolated on 2.5% agarose gel. *Actb* mRNA is detected as an internal control. (C)-(D) The inclusion percentage of exon 9* is the sum of the upper and lower band strengths divided by the upper and lower band strengths. **P*<0.05, ***P*<0.01 unpaired t-test.

3. Ibrutinib induces the expression of $Ca_V1.2$ channels and increases K^+-tiggered $[Ca^{2+}]_i$



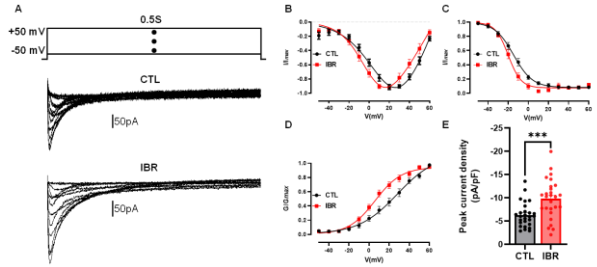
(A)-(C) The membrane expression of $Ca_V1.2$ was detected by Western blotting in NRVMs and heart tissues from control and Ibrutinib-treated, Na-K ATPase protein was detected as internal control. The relative band densities were analyzed and normalized to Na-K-ATPase. (D) NRVMs were treated with ibrutinib, $[Ca^{2+}]_i$ was measured with Fluo-4AM, and fluorescence intensity was monitored under confocal microscopy using time-series scanning patterns. (E) The $\Delta[Ca^{2+}]_i$ fluorescence intensity is measured by dividing the change in the fluorescence signal by the average resting fluorescence. ***P*<0.01,****P*<0.001 2-way ANOVA followed by post-hoc test.

4. Ibrutinib prolongates the cardiac action potential duration



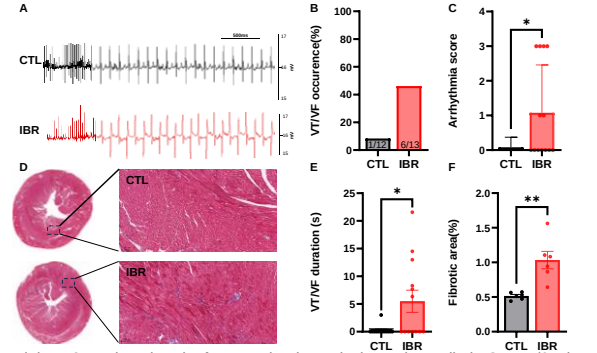
(A) Representative records of AP untreated (black) and ibrutinib treatment (red) in NRVMs. (B) NRVMs resting film potential and AP amplitude values. (C) AP uplink speed (dV/dt) value of NRVMs. (D) APDs at 30%, 50%, 90% of membrane repolarization for NRVMs. Each dot represents the value from 1 cell. ***P*<0.01, #*P*<0.0001, unpaired t-test

5. Ibrutinib shifts the window currents of $Ca_V1.2$ channel to hyperpolarization in NRVMs



(A) $Ca_V1.2$ channel current was recorded at different test potentials increasing from -50 mV to 50 mV. With 5 mmol/L Ca^{2+} as external solution, 10-mV was increased at each step in NRVMs with or without ibrutinib treatment. (B) The plots of the I-V curve is derived from the I-V protocol plot recorded in NRVMs that are not processed (black) or treated with ibrutinib (red). (C-E) According to the $Ca_V1.2$ channel activation and deactivation curves of NRVMs treated with different treatments, the current densities of $Ca_V1.2$ channels in NRVMs treated with untreated and ibrutinib were given. ****P*<0.001 unpaired t-test.

6. Ibrutinib increases cardiac arrhythmia susceptibility and tissue fibrosis



(A) An 8-week outbreak of unsustained ventricular tachycardia in C57BL/6 mice treated with ibrutinib (25 mg/kg/day). (B, C, E) The incidence of ibrutinib in untreated and treated mice, the score that determined the occurrence of arrhythmia, and the duration of unsustained ventricular tachycardia. (D) Representative images of Masson staining of untreated and post-ibrutinib treated hearts. (F) Percentage of cardiac fibrosis area. Scale=100μm. **P*<0.05, ***P*<0.01 unpaired t-test.

Conclusions

These findings suggest that Ibrutinib could increase $Ca_V1.2$ expression of myocardial cells, and shifted the *I-V* curve of $Ca_V1.2$ channels towards hyperpolarization. Thus, this causes intracellular calcium signaling disturbance and triggering cardiac arrhythmia.