Title: The Effect of Isoliquiritigenin on the HERG Potassium Channel

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Research Rationale: Xin Su Ning (XSN) is an herbal anti-arrhythmic medicine formulated with 11 Chinese herbal medicine. It has been shown to prolong action potential duration and to block potassium and sodium channels of isolated cardiac myocytes, which implies the property of class I & III anti-arrhythmic medicine. Isoliquiritegenin (ISL) is a constituent compound of XSN that has been shown to have anti-inflammatory, anti-angiogenic and anti-cancer activities. In this study, we aim to extend the current understanding of the anti-arrhythmic mechanism of XSN by identifying any effect of ISL on the hERG potassium channel.

Methodology: Electrophysiological assays were conducted with Axopatch 200B patch clamp system in a Chinese hamster ovarian (CHO-K1) cell line stably transfected with the human isoform of the hERG channel. Solutions were prepared as previously described[1]. Drug was perfused to cells at room temperature. ISL at various concentrations were applied to the cells and the effects on the peak channel amplitude were measured. Furthermore, the effects of ISL on activation and deactivation kinetics of the hERG channels were studied respectively by applying a range of voltage clamp protocols to assess the binding property of ISL on hERG Channels.

Results: ISL inhibited the amplitude of the maximum hERG channel potassium current in a dose-dependent manner. ISL blocks hERG in a bi-sigmoidal fashion; the sigmoid at low concentrations (fig.1A) has EC50 0.16309 ± 0.02832 and at high range (fig.1B) has EC50 8.53946 ± 2.06073. The bi-sigmoid suggests ISL binds to both the oestrogen site (0.01-1µm) and channel pore site (1-400µm). ISL caused a significant leftward shift of the activation curve (fig.1C), indicating that the compound binds to the channel in the open state; there was no statistically significant difference in the deactivation kinetics of the channel (fig.1D). ISL reduced the current within a wide range of membrane potentials -80 to +50mV (fig.1F)

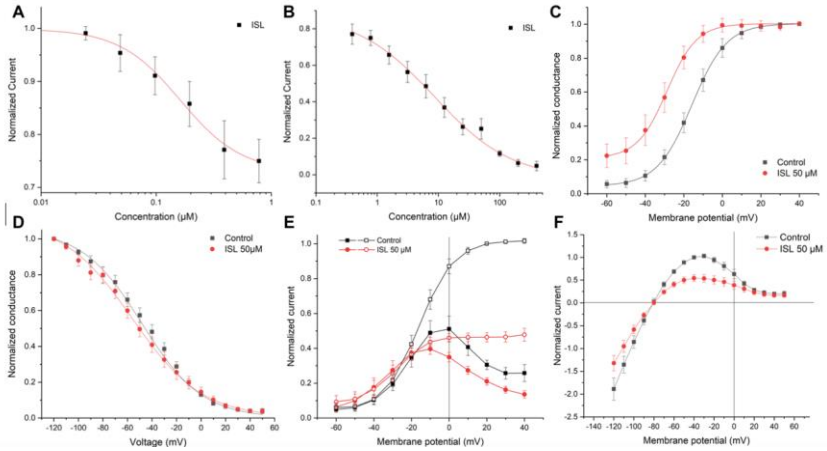

Fig. 1. Acute effects of ISL on hERG current in CHO-K1 cell line. (A and B) dose-response curves of the inhibition on human hERG channel by ISL at low range and high range (n=6). Normalised and averaged current values for each concentration plotted to produce a dose-response curve fitted with the Hill Equation. The inhibitory effect of ISL was reversed with washout. (C and D) Normalised current values were plotted and fitted with the Boltzmann equation to show effects of 50µm ISL on activation and deactivation kinetics (n=8). Derived from averaged I-V curves of the tail currents generated by 'activation' and 'inactivation' pulse protocols. (E and F) Normalised and averaged I-V relationships. I-V curves in (E) are for the maximal tail currents at repolarisation potential of -55mV (open symbols) and for currents at the end of the depolarising pulse (solid symbols) against the pulse potential. In (F) the I-V curves are for the maximal repolarisation-evoked tail currents against repolarisation potential.

Conclusion: ISL blocks hERG channel in a dose-dependent manner with affinity for the activated state. Any drug that blocks hERG channels would be expected to prolong action potential duration of the cardiac myocytes. Our results implies that ISL would contribute to the class III antiarrhythmic action of XSN as one of the active components. Further study into the anti-arrhythmic actions of XSN could prove beneficial in understanding the action of multi-component anti-arrhythmic drugs.

[1]Ma, Y et al. Ion channel targeted mechanisms of anti-arrhythmic Chinese herbal medicine Xin Su Ning. *Frontiers in Pharmacology* **10**, 70 (2019).