Assessment of Multiple Positive Controls in the Langendorff Isolated Heart Assay and How They Translate to Whole Animal

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1 INTRODUCTION

The ex vivo Langendorff system utilizes the isolated intact heart and provides measurements of chronotropy, inotropy, electrophysiology, and coronary perfusion flow rate. The assay is independent of systemic influences such as sympathetic, parasympathetic, and non-heart-related metabolism unlike the whole animal implanted telemetry assays.

The current study assessed the effects of positive controls Isoproterenol (ISO), Atenolol, Ranolazine, and Diltiazem across both assays for comparison. Cardiovascular endpoints assessed include left ventricular pressure (dP/dt+), end diastolic developed pressure (EDP), electrocardiography, heart rate (HR), body temperature, and arterial blood pressure. The results from this study further validate the translation between indices of contractility and changes in ECG intervals from isolated heart to whole animals. Utilizing these low cost, short duration with minimal test compound models earlier in the drug development process may identify cardiovascular function changes that manifest in whole animal (preclinical) and human (clinical) with less test compound required.

2 METHODS

For the ex vivo Langendorff assay, Buprenorphine was administered at least 25 minutes prior to anesthesia induction at 0.05 mg/kg subcutaneously, also, an injection of 600 units of heparin [1000 United States Pharmacopoeia (USP) units/mL] was administered by intraperitoneal (IP) injection. Animals were anesthetized to a surgical plane using isoflurane at 2 to 3% with oxygen as the carrier gas. After ensuring that the animal was completely anesthetized via toe and abdominal pinch, a bilateral carotid arteriotomy combined with transverse elemyoty and pericardial incision was made for arterial and venous access in order to introduce the cardioplegic solution.

25 male Hartley guinea pig hearts were used; five hearts served as a vehicle control with Modified Krebs-Henseleit (MKH) solution at physiologic temperature (37°C) by retrograde perfusion. Two flexible unipolar electrodes were placed on the heart, one over the epicardium of the left ventricle and the other over the epicardium of the right atrium, emulating a Lead II electrocardiogram. A fluid-filled cannulated balloon, connected to a pressure transducer, was inserted into the left ventricle. The balloon was filled with deionized water to conform to the endocardium of the ventricle and to achieve a starting end diastolic pressure of between 1 and 10 mmHg. All pressure and electrocardiogram data was collected continuously using IOX Data Acquisition System from Emka Technologies (Paris, France).

For the in vivo telemetry protocol, the radiotelemetry system (Data Sciences International, St. Paul, MN) consisted of HDS21 series radiotelemetry transmitters (with capabilities to collect left ventricular pressure, body temperature, and electrocardiographic waveform) receivers (RM-1), and 1 or more data exchange matrices (DEM) that relayed information from the transmitter to the computer. An ambient pressure reference monitor (APR-1) was coupled to the DEM to measure the barometric pressure and provide a digital signal to the DSI PONEMAH system. The DSI PONEMAH system uses the waveforms provided by the APR-1 to correct pressure measurements obtained from the implant for changes in atmospheric pressure. The hardware connected to the Datquest OpenART Acquisition Interface provides direct digital signals to the DSI PONEMAH software. The ECG and arterial waveform and body temperature

3 METHODS cont.

data were recorded and analyzed by the DSI PONEMAH data acquisition software, version 5.0. ECG and arterial pressure waveforms were sampled at 500 Hz. Temperature data was sampled at 50 Hz. Data acquired continuously were logged every 120 seconds. During the data processing, the logging rate was changed to 1 Epoch. All data were analyzed using ECGAuto (Emka Technologies, Paris, France).

Four telemetry animals were used to assess cardiovascular parameters in vivo (n=4). Prior to each guinea pig dose, at least 1 hour of baseline was collected and there were injected subcutaneously with 0.1 mg/kg of isoproterenol HCl. Telemetry parameters were collected for at least 4 hours and analyzed to assess the pharmacodynamics response to the test article. The target pharmacodynamics response was a heart rate change of at least 10% and an increase in dP/dt+ of at least 500 nV/mV. Atenolol was administered via oral gavage at 50 mg/kg followed by a 3 mL flush with DI water and the telemetry parameters were collected for a minimum of 12 hours. Ranolazine was administered via oral gavage at 30 mg/kg followed by a 2 mL flush and telemetry parameters were collected for a minimum of 24 hours. Diltiazem was administered subcutaneously at 15 mg/kg and the telemetry parameters were collected for a minimum of 24 hours.

4 RESULTS

Isoproterenol: Heart rate (HR) and dP/dt+ increases across all concentrations in the isolated heart as well as telemetry when compared to baseline.

Atenolol: Developed pressure mean (DP) decreased across concentrations in the isolated heart and telemetry, compared to baseline. Left ventricular pressure (dP/dt+) decreased across concentrations in the isolated heart. Telemetry data showed an initial decrease in dP/dt+ with subsequent increase to homeostatic baseline conditions.

Ranolazine: QTcV mean and J-T Peak increased across all concentrations in the isolated heart. HR decreased across concentrations for the isolated heart. Telemetry showed an initial decrease in HR before increasing to homeostatic conditions when compared to baseline. Diltiazem: HR decreased across concentrations for the isolated heart. Telemetry showed an initial decrease in HR before increasing to homeostatic conditions when compared to baseline. dP/dt+ decreased across all concentrations in the isolated heart as well as telemetry when compared to baseline.

5 DISCUSSION

The cardiovascular effect observed in the isolated heart translated well into the whole animal telemetry model, both expressing the expected clinically reported effects with the exception of Ranolazine. Ranolazine blocks both late NA and IKr channels with similar IC50 values, 7.9 µM and 8.03 µM, respectively. Late NA block increases the rate of repolarization and should decrease the interval length of JT related to QTc. The effects on JT represented in these results are the sum of late NA block, WK block and a decrease in heart rate (figure not shown). The translation between models further validates the use of the Langendorff for cardiovascular testing in drug development and is particularly useful in the discovery stages to screen multiple candidate drugs for off target liabilities. Early detection of potential liabilities aid in de-risking strategies, providing a means to rank candidates based on cardiovascular effects.