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 nonheart-related metabolism unlike the whole animal implanted telemetry assay.

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 (DP)], 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

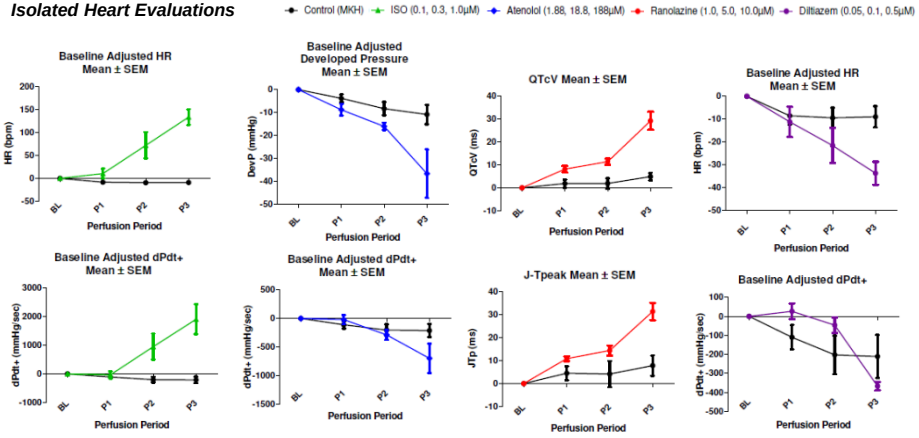
For the ex vivo Langendorff assay, Buprenorphine was administered at least 25 minutes prior to anesthesia induction at 0.05 mg/kg subcutaneously; also, an injection of 600 units of heparin [1000 United States Pharmacopoeia (USP) units/mL] was administered by intraperitoneal (IP) injection. Animals were anesthetized to a surgical plane using isoflurane at 2 to 5% with oxygen as the carrier gas. After ensuring that the animal was completely anesthetized via toe and abdominal pinch, a bilateral anterolateral thoracotomy combined with transverse sternotomy and pericardiotomy was performed. Excised hearts were arrested and preserved in cold cardioplegic solution.

25 male Hartley guinea pig hearts were used; five hearts served as a vehicle control with Modified Krebs-Henseleit (MKH) The isolated hearts were perfused with vehicle with a 20 minute equilibration period, followed by a 20 minute baseline collection, and then perfused for approximately 20 minutes with either vehicle or increasing concentrations of formulated test article. Perfusion began at a constant flow of approximately 15 mL/min and changed to constant pressure targeting approximately 60 mmHg after cardiac parameters stabilized. The animals used were approximately 6 weeks of age and weighed between 300 g and 600 g at the initiation of dosing.

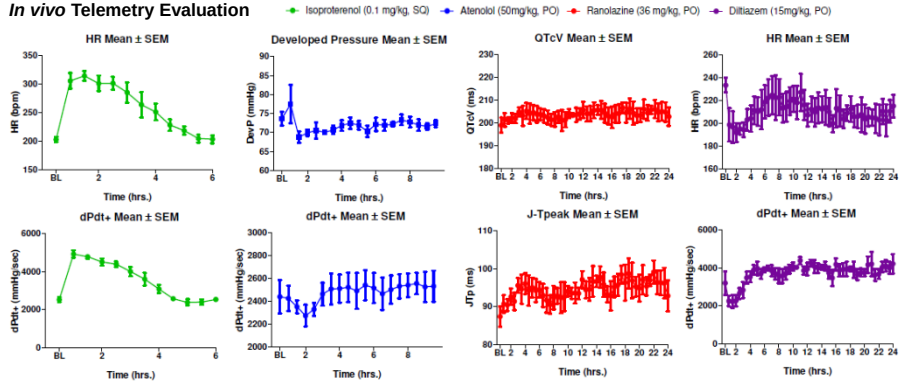
Guinea pig hearts were cannulated via the aorta and perfused at a constant pressure of 60 mmHg with oxygenated Modified Krebs-Henseleit (MKH) solution at physiologic temperature (37°C) by retrograde perfusion. Two flexible unipolar electrodes were placed on the heart, one over the epicardium of the left ventricle and the other over the epicardium of the right atrium, emulating a Lead II electrogram. 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 telemetered assay, the radiotelemetry system (Data Sciences International, St. Paul, MN) consisted of HDS21 series radiotelemetry transmitters (with capabilities to collect at minimum arterial pressure, body temperature, and electrocardiographic waveforms) receivers (RMC-1), and 1 or more data exchange matrices (DEM) that relayed information from the receivers 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 measurements provided by the APR-1 to correct pressure measurements obtained from the implant for changes in barometric pressure. The hardware connected to the Dataquest OpenART Acquisition Interface provides direct digital signals to the DSI PONEMAH software. The ECG and arterial waveform and body temperature

Isolated Heart Evaluations



***In vivo* Telemetry Evaluation**



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 telemetered 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 then 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 were a heart rate change of at least 10% and an increase in dP/dtmax of at least 500 mmHg/sec. 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 36 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 dPdt+ 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 assay. Telemetry data showed an overall slight decrease as well compared to baseline. Left ventricular pressure (dPdt+) decreased across concentrations in the isolated heart. Telemetry data showed an initial decrease in dPdt+ with subsequent increase to homeostatic baseline conditions.

Ranolazine: QTcV mean and J-T Peak increased across all concentrations in the isolated heart. Telemetry data showed an overall slight increase in QTcV and J-T Peak as well 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. dPdt+ 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_{peak} but instead shows an increase similar in magnitude to QTc. The effects on JT_{peak} represented in these results are the sum of late NA block, IKr 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.