Comparison of in vitro and clinical in vivo effects of pimobendan a canine heart failure drug


1. INTRODUCTION

High throughput in vitro systems that gauge inotropic and lusitropic effects on HPS-CMs at controlled beat rates are expected to have a significant impact on cardiovascular safety and drug discovery targeting heart failure. Impedance recordings of HPS-CMs have been validated for the detection of inotropic drugs (Scott et al., 2014). The assay shows sensitivity and specificity for inotropic drugs comparable to the study done in adult dog cardiomyocytes using the myoStile system, a geometry measurement system that measures changes in sarcomere length (Harmer et al., 2012).

The contractile event in the impedance recording is the impedance twitch, a complex waveform consisting of one negative and one or two positive peaks depending on the cell culture conditions. Here we dissected pharmacologically the impedance twitch component associated with excitatory calcium entry using Myosin II inhibition and the excitation-contraction coupling component using the calcium channel agonist FPL64176. After defining the impedance parameters underlying cell contractility we investigated the extent to which the impedance assay compares with the animal model by using the cardiac sarcomere preparation that is currently used to assess heart failure in veterinary medicine.

The evidence indicates that the impedance twitch is a good surrogate of the belch contraction and can be used to assess contraction and in general excitation-coupling effects during drug development. The assay was able to detect the contractile effects of pimobendan at customary screening concentrations.

2. METHODS

We used the CardioECR instrument (Acea Bioscience) to record impedance and esedricular field potentials (EFP) and paced the cells. HPS-CMs (GFP) were from Caldaris Dynamics International. Cells were cultured in 49 well plates and paced at 6.7 Hz.

The impedance twitch features motion and excitation-contraction coupling mechanisms.

The output of the instrument is the Cell Index, a measure of the electrode impedance relative to the background reading. During analysis values were transformed to ohms according to 2*4-cx+c*10^2. Background was analyzed using Matlab and macros written in R.

Four naïve male Beagle dogs were instrumented with Data Science International (DSI) Physical Dynamic (DP) implants equipped with high density pressure and ECG leads fixed to the left clavicle, Heart Rate, LV parameters (dP/dt max, left dP/dt, systolic arterial pressure, QA interval duration and arterial impedance (HR, QRS, QT and QTc) were collected for 24 hours pre-treatment for baseline assessment and 3 hours post dose for 24 hours post dose (DOR Pharmacal software). Animals were group housed in European (EU) pens and received vehicle or 3 dose levels of Pimobendan (0.1, 0.3 and 1 mg/kg) as per modified cross-over design.

The positive and negative peaks of the impedance twitch were 0.72 ± 0.03 Ω and 6.5 ± 0.3 Ω (mean ± sem, n=12). Twitch duration and total twitch area were 425 ± 7 Ωms and 1495 ± 70 Ωms, respectively. Maximal rates of contraction (+dP/dtmax) and relaxation (-dP/dtmax) were 12 ± 2 Ωms and 38 ± 12 Ωms respectively.

3. RESULTS

All components of the impedance twitch occur after the sodium spike of the cardiac action potential.

To define the different components of the impedance twitch it is necessary to assess its waveform in the context of the cardiac action potential. This can be accomplished by recording EFP simultaneously. Figure 1A shows the organization of the impedance and EFP electrodes in the CardioECR plate. Figures 1B and C shows EFP and impedance recordings from one well paced at 6.7 Hz. Note that 1) the negative deflection of the impedance twitch occurs after the sodium spike vanished indicating that it is not associated with local changes in membrane potential, 2) impedance twitch relaxation is a two exponential phenomenon and 3) most of the impedance twitch occurs during the active phase of the cardiac action potential.

Pimobendan modulates the motion-dependent component of the impedance twitch.

Pimobendan is a calcium sensitizer and phosphodiesterase II and IV inhibitor used in veterinary medicine to treat heart failure (Hsiao & Sykes, 2015). Figure 4A shows that pimobendan up to 10 µM does not affect the waveform of the EFP. Figure 4B shows the concentration and time dependent effect of phosphodiesterase. After one hour exposure peak amplitudes (+dP/dtmax and -dP/dtmax) increased significantly by 12% and 10% respectively compared to control. Pimobendan (10 µM) had no effect on the negative peak amplitudes (+dP/dtmax).

Pimobendan increases contractility in group housed dogs.

The positive values for +dP/dtmax, -dP/dtmax and QA interval were respectively (mean ± sem, n=4): 4737 ± 141 mmHg/s and 113 ± 1 ms. Administration of Pimobendan induced a dose-dependent increase in +dP/dtmax reaching a maximum increase after 1.5 hours exposure at 1 mg/kg (Figure 5). The effects were paralleled by a 15% reduction in the QA interval consistent with an effect in contractility and a 19% increase in -dP/dtmax at 1 mg/kg from 1 to 3 hours post dose that can be partially explained by an increase in heart rate (not shown). The mean increase in +dP/dtmax of 5950 mm/s in the control group was comparable with previously published data (107% change, Markert et al., 2003) over a 7-hour period.

4. CONCLUSIONS

Pimobendan is a good surrogate of the belch contraction and can be used to assess contraction and in general excitation-coupling effects during drug development.

In vitro impedance screening of HPS-CMs at customary screening concentrations correlated the contractility effects of pimobendan.

REFERENCES


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Miosyn II inhibition isolates the motion-dependent component of the impedance twitch.

Beat-based isolation of the motion-dependent component of the impedance twitch.