Evaluation of Clinically-Relevant Proarrhythmic ECG Biomarkers in Conscious Beagle Dogs, Using a Newly Developed Multiple Lead ECG Telemetry Implant

Chris Regan[1]; Tom Delahanty[2]; Bob Brockway[3]; Pat Fanelli[1]; Hillary Regan[1]; Ted Detwiler[1]; Shaun Gruver[1]; Gary Miller[2]; Frederick Sannajust[1]

[1]Department of Safety & Exploratory Pharmacology, SALAR Division, MRL Laboratories (West Point, PA), Merck & Co., Inc., Kenilworth, NJ, USA; [2]TSE Systems, Bad Homburg, Germany; [3]VivaQuant Inc., St Paul, MN, USA.

Abstract

Preclinical proarrhythmic risk assessment of multiple ion channel antagonists has mainly focused on in silico and in vitro safety pharmacology (SP) models. Continued efforts to develop preclinical in vivo tools are essential to provide better clinical translatability and enable a more comprehensive assessment of ECG morphology through collection of multiple cardiac axes and vector magnitude leads consistent with proposed clinical methods. As such, we developed and conducted proof-of-concept (POC) evaluation of a Stellar/TSE Systems telemetry implant to simultaneously record limb and precordial ECGs and arterial blood pressure (BP). Augmented ECG leads (aVR, aVF, aVL) were also calculated in real time, resulting in continuous 8-lead ECG data. Briefly, beagle dogs (N=5) were instrumented with 6 subcutaneous electrodes to record leads I, II, III, V1, and V4 and a solid-state transducer to record arterial BP. Animals were then orally administered vehicle, dofetilide (DOF, 30 µg/kg), or DOF + verapamil (DOF + VER, 30 µg/kg + 5 mg/kg), to determine the effects of pure or mixed ion channel blockade on rate-corrected QT and J-Tpeak (QTc and JTpc, respectively) and Tpeak-Tend (TpTe). Vehicle/control studies demonstrated POC to collect continuous 8-lead ECG with arterial BP waveforms. DOF oral administration resulted in similar lead II peak increases from baseline in QTc (22 ± 3 msec) and JTpc (22 ± 2 msec) and a mild increase in TpTe (2 ± 0.4 msec) 1-3 hr postdose. DOF + VER oral administration attenuated the 1- to 3-hr postdose peak QTc and JTpc increases by 5 msec and 10 msec, respectively, with no effect on TpTe. Five to 6 hr post-DOF dosing, increases were observed in both JTPc (11 ± 3 msec) and QTc (13 ± 3 msec). Similar QTc increase 5-6 hr postdose were observed in the DOF/VER-treated animals, but there was only a slight JTPc increase (3 ± 2 msec). Interestingly, DOF/VER resulted in similar but more sustained TpTe increases for up to ~8 hr postdose. While the preclinical value of using precordial and/or vector magnitude leads over single-lead ECG in telemetered animals requires further investigation, these data demonstrate a preclinical platform by which current and proposed clinical ECG analysis methods can be better employed preclinically to assess pro-arrhythmic risk. Moreover, implementation of combined, telemetered frontal and transverse ECGs should enable enhanced ECG morphology assessments in SP, toxicology, and/or combined SP/toxicology studies.

METHODS

- Male beagle dogs (6-10 kg, N=5) were instrumented with Stellar/TSE telemetry implant. Novel Stellar/TSE implants were designed specifically to record simultaneously 5-lead ECGs: Lead I, II, III, and 2 precordial leads that were implanted as V1 and V4. The calculation of augmented leads aVR, aVF, and aVL was completed in real time within the acquisition software. This provided the capability to continuously record 8-lead ECGs in conscious, freely moving, telemetered animals and arterial BP, along with body temperature and activity. ECG leads were placed subcutaneously, the arterial BP sensor was introduced into the abdominal aorta via the femoral artery, and the implant body was secured subcutaneously
- Following post-surgical recovery, animals were orally administered (PO) the following vehicle or test agents:
  - **0.5% methylcellulose (0.5%MC, vehicle):** 5 mL/kg, N=5
  - **Dofetilide (hERG blocker):** 0.03 mg/kg, N=5
  - **Verapamil (Ca++ channel blocker):** 5 mg/kg, N=5
  - **Mexiletine (Na+ channel blocker):** 7.5 mg/kg, N=5
  - **Dofetilide + verapamil:** 0.03 mg/kg + 5 mg/kg, N=5
  - **Dofetilide + mexiletine:** 0.03 mg/kg + 7.5 mg/kg, N=5
- Data were collected from ~2 hr prior to dosing to ~20 hr postdose. Raw waveforms were recorded using Notocord-HEM® (v 4.3.0.77) software. Six studies were conducted, with a ~1-week washout period between each treatment
- ECG interval analysis was conducted using VivaQuant RhythmExpress® (RE) software on either single lead (Lead II) or vector magnitude (VM) leads. VM leads were calculated in RE as the square root of the sum of squares using leads I, II, and III (VM II I III) or leads I, II, and V1 (VM II I V1) after filtering channels to maintain stable zero baseline. Data were reported as beat-to-beat or binned in 15- or 60-min time periods within animal and study, then averaged to determine mean ± SE
- Representative lead II and calculated VM ECG leads with marking

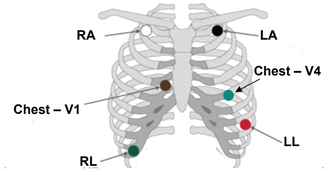
- Baseline ECG interval data from vehicle study (N=5)

Lead	Test Agent	N	HR bpm	SE	QRS msec	SE	QT msec	SE	QTcV msec	SE	JTpc msec	SE	TpTe msec	SE
Lead II	Vehicle	5	101	1.2	34	0.7	218	2.8	245	1.2	187	2.8	35	0.6
VM II I III	Vehicle	5	101	1.1	36	1.1	219	2.9	246	1.6	185	2.1	36	0.5
VM II I V1	Vehicle	5	101	1.1	39	0.7	220	3	248	1.4	183	2.9	36	0.4

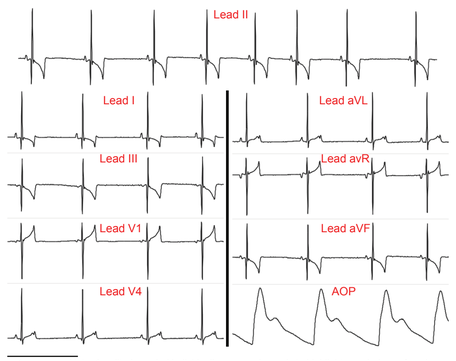
RESULTS

Stellar/TSE 5-Lead ECG Implant With BP

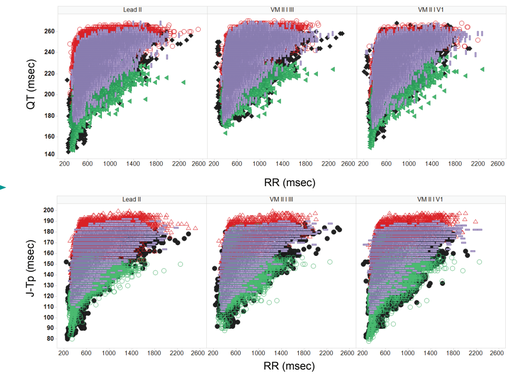
5-Lead ECG Placement



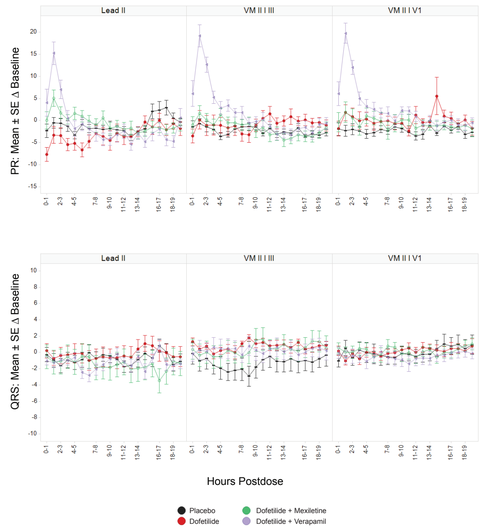
Continuous 8-Lead ECG + Arterial BP

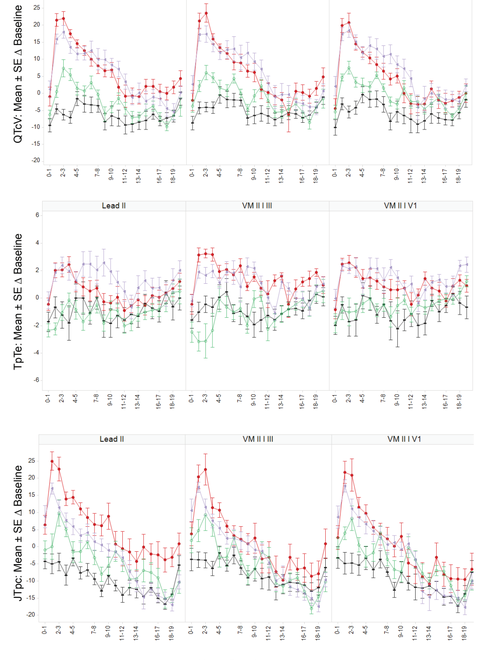


Beat-to-Beat QT or JTp vs RR: 30 min of Continuous Data Selected Within 1-4 hr Postdose



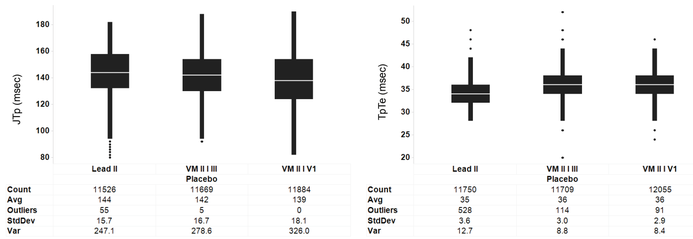
- **Dofetilide:**
  - Upward shift: QT and JTp plots
- **Dofetilide + verapamil:**
  - JTp shift attenuated
  - Mild/no effect on QT
- **Dofetilide + mexiletine:**
  - JTp shift attenuated
  - QT shift attenuated





- Marked, dofetilide-dependent increases in QTcV, JTpc, and Tp-e
- Slight attenuation of JTpc in dofetilide + verapamil group vs dofetilide alone. No effect on TpTe increase
- Mexiletine + dofetilide resulted in decreases in QTcV, JTpc, and TpTe
- Verapamil-dependent PR prolongation. No effect on QRS with any test agent
- Small differences among measures from different leads suggest potential areas of refinement to increase sensitivity. Further work will be required

Box Plot: ~30 min of Beat-to-Beat Lead II or VM Lead Data: JTp and TpTe



	JTp (msec)			TpTe (msec)		
	Lead II	VM II I III Placebo	VM II I V1	Lead II	VM II I III Placebo	VM II I V1
Count	11526	11689	11884	11750	11709	12055
Avg	144	142	139	35	36	36
Outliers	55	5	0	528	114	91
StdDev	15.7	16.7	18.1	3.6	3.0	2.9
Var	247.1	278.6	326.0	12.7	8.8	8.4

- Fewer outliers using VM leads for JTp and TpTe. Inclusion of V1 reduced JTp outliers to zero
- Additional data analysis required to determine optimal leads/conditions to create VM lead

CONCLUSIONS

- We developed novel Stellar/TSE implants to record up to 5 ECG leads, including precordial leads (with the ability to calculate the 3 augmented leads online), along with arterial BP, body temperature, and activity
- Our data demonstrated ability to accurately measure and determine compound-dependent differences in enhanced ECG intervals JTp and TpTe to better understand pro-arrhythmia in telemetered animals
  - Results from combined dosing of “pure” ion channel blockers were in line with previously published clinical data, suggesting the translational value of the model
  - Results demonstrated ability to derive stable, reliable VM leads from freely moving, telemetered animals
- The inclusion of precordial leads, alone or as part of a vector magnitude, may enable better assessment of enhanced ECG biomarkers, JTp, and, particularly, TpTe, given the potential value of adding one or more horizontal axes to the analysis

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