Model Establishment for Cardiovascular Evaluation Using the Novel Stellar TSE's Type PPBTA-XL Telemetry Transmitter in the Vervet (St. Kitts Green Monkey)

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Objectives

This study was conducted to evaluate the utility of the Stellar TSE's Type PPBTA-XL Telemetry Transmitter for cardiac function measurements in St. Kitts green monkeys. Specific objectives included:

- To establish and confirm the surgical procedure required to implant TSE telemetry transmitters in the St. Kitts African green monkeys
- To test and modify and best adapt the design of the implanting catheter to best fit the test system so as to obtain optimal data
- To gather baseline cardiac activity data from telemetered monkeys and evaluate changes induced by a positive control
- To confirm the use of this test system in combination with the novel Stellar TSE's Type PPBTA-XL Telemetry Transmitter for further cardiac functioning and safety assessment studies.

Background

The St. Kitts green monkey, or vervet (*Chlorocebus sabaeus*) has been widely used in biomedical research, but application of the species to safety pharmacology is not as extensive and well characterized as rhesus or cynomolgus macaques. With greater reliance on nonhuman primates, which share tighter genetic, physiologic and anatomic homology to humans, for preclinical safety evaluations of biologics and gene therapy strategies there is increasing demand for nonhuman primate test systems for cardiovascular safety assessments, and the value of alternative species and telemetry platforms offering data quality and cost advantages.

Wireless transmission of key physiological parameters such as blood pressure (BP), electrocardiography (ECG), activity and body temperature in freely moving monkeys provides the best possible experimental condition to study regulation of blood pressure, including the effect of pharmacological interventions and social interaction.

In this study the Stellar TSE's Type PPBTA-XL Telemetry device was tested in St. Kitts African green monkeys for potential use in cardiac safety monitoring.

Stellar TSE's Type PPBTA-XL Telemetry Transmitters Specifications and Capabilities:

- Solid state pressure sensor with higher fidelity and response bandwidth compared to fluid filled catheters
- Programmed scheduled and/or continuous recording
- Autonomous operation outside antenna range (model dependent)
- Cross species/body weight technology on the receiver side - one system talks to all implants
- Mobility of the system receiver/antenna coupled to laptop
- All digital implant technology allows for group housing of animals with no limitations

Methods

This study was conducted in compliance with facility SOPs and AAALAC standards for the use of animals in biomedical research following IACUC approval. Eleven (11) adult males ranging in weight from 5-7 kg were enrolled in the study. The staged study design that permitted refinement of data acquisition, implantation procedures and catheter design and placement are detailed in **Table 1**. Telemetry data acquisition was conducted at progressive stages of test system optimization. At data acquisition time points systemic blood pressure (BP), left ventricular pressure (LVP), heart rate (HR) and electrocardiogram (ECG) parameters were determined. Systemic BP, LVP and HR are the cardinal parameters discussed here.

Fifteen seconds of telemetry data were collected every 30 minutes for up to a 48 hour period at each data collection interval. This data has been averaged in 6 hour intervals to facilitate review of the data. To conduct assessments monkeys remained unsedated in their home cages. Analysis of data was handled using the software accompanying the TSE system (Acqknowledge Ver. 4.4, BIOPAC).

Figure 1: Screenshot of the double hump near the top of the LVP wave

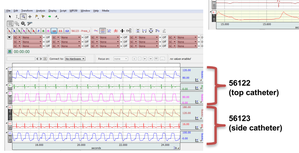

Figure 2A: Screenshot of the clean LVP wave form observed in the trial 5 animals (3 days and 3 hrs post surgical implantation for 56122 and 56123 respectively).

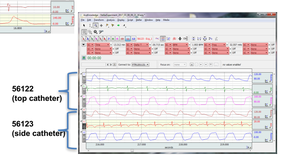

Figure 2B: Screenshot of the clean LVP wave form observed in the trial 5 animals (13 and 8 days post surgical implantation for 56122 and 56123 respectively).

TABLE 1: STAGED TELEMETRY CATHETERS TESTING AND OPTIMIZATION

BETA TEST STAGES	ANIMAL #	TELEMETRY DEVICE #	CATHETER SPECIFICATIONS	ADDED FEATURES	ISSUES ENCOUNTERED
1	A112, A088, A092	56031, 56032, 56033	Catheter length from suture grove to tip = 5 mm; Sensor position: At tip		At implantation signals were OK but a small "double hump" at the top of the LVP wave was evident in some monkeys. LVP signals were initially stable but over time a "drift" in LVP was observed whereby the LVEDP progressively got higher and higher (away from 0) and the LVESP also got higher and higher. Thus the entire LVP graph was shifted upwards. The "double hump" appeared to be a second contraction of the heart at the top of the LVESP wave became more obvious over time. (Fig. 1)
2	K763, K785	56103, 56104	Catheter length from suture grove to tip = 5 mm; Sensor position: At tip	Same as above but wanted to check if it the drift happened by chance.	"Double hump" was still observed and the LVP drift was still evident.
3	K597, K330, T964	56118, 56119, 56120	Catheter length from suture grove to tip = 5 mm; Sensor position: At tip	Tip changed from flexible to more sturdy to overcome the possibility that the flexibility in the catheter facilitated it being trapped by the papillary muscles during insertion.	"Double hump" was still observed and the LVP drift was still evident. In some instances, the LVESP and LVEDP gave the same readings.
4	K959	56121	Catheter and sensor same as the second model with a sturdier tip	A hard cap housing the sensor was attached at the tip of the catheter to prevent bending during insertion.	The cap was too hard thus insertion would require a much larger incision at the apex of the heart. When tunneling the LVP catheter toward the heart using a trochar during surgery, the hard tip (which was too big) broke off. Thus the LVP catheter was not implanted so no LVP data was collected from this monkey.
5	(a) A169, (a) A403	(a) 56122, (b) 56123	Catheter length from suture groove = 15 mm; Sensor position: (a)at the tip (b)on the side	Marker to identify the position of the side sensor (b) to allow orientation of the sensor toward the septum to keep it away from the papillary muscles.	

Preparation for telemetry and vascular access port (VAP) implantation:

- Following baseline screening, animals underwent cardiac telemetry and VAP implant surgery. Monkeys were fasted overnight and anesthetized with ketamine (10 mg/kg, IM), followed by atropine (0.04 mg/kg, IM). Meloxicam was administered at a dose of 0.2 mg/kg, IM, pre-operatively.
- Flo-cillin (50,000 U/kg, I.M.) was also administered once pre-operatively on the morning of surgery.
- The monkeys were shaved and prepped for aseptic surgery to include a left thoracotomy, an off-midline laparotomy, a jugular cut-down and left dorsal lateral thorax placement of a side access VAP.
- The monkeys were then intubated and maintained on anesthesia with isoflurane, to effect with supplemental heat via a circulating water blanket.
- Heart rate, respiratory rate, rectal temperature, ECG, SPO_2, CRT and mucous membrane color were monitored during the procedure.
- A peripheral catheter was placed for administration of lactated Ringer's solution at a rate of approximately 10 mL/kg/hr.

Telemetry implantation surgery: Prior to implantation, each study monkey was assigned a specific transmitter unit. An off midline laparotomy was performed per animal and the systemic pressure catheter advanced from the internal iliac to the mid aorta. The transmitter body was anchored to the abdominal wall and the bio-potential leads and LVP catheter were tunneled to the left thorax. A left thoracotomy was performed and the LVP catheter implanted and anchored via the ventricular apex. The bio-potential leads were anchored to the epicardium for epicardial ECG measurement.

VAP Placement for Isoproterenol administration: A midline neck incision was made and the left internal jugular exposed and isolated. Via a veinotomy a 4-6 Fr rounded tipped tapered polyurethane catheter (Taper Cath™ polyurethane catheter) was inserted caudally, approximately 5.0 cm, and anchored into place with non-absorbable suture. The distal and proximal aspects of the vein was then ligated and the catheter flushed with normal saline. The catheter was then tunneled to the thoracotomy incision, trimmed to an appropriate length and attached to a side access port (Cath-In-Cath 2 Port System). The length of the catheter remaining with the monkey and the dead space was noted. A pocket was formed under the edge of the latissimus dorsi and the port anchored with non-absorbable suture to the underlying muscle bed. The catheter and port were then locked with TCS® (taurolidine-citrate) locking solution (Access Technologies, Skokie, IL).

Final Closure: All muscle groups were apposed with an appropriately sized absorbable suture. All fascia and skin incisions were closed with an appropriately sized absorbable suture. The lungs were hyper inflated and the chest tube removed at the end of the procedure and the skin closed appropriately with an appropriately sized absorbable suture. Tissue adhesive was applied when appropriate. Bupivacaine was infused into the incision sites to minimize local pain and discomfort. Buprenorphine 0.02 mg/kg was administered IM TID X 3 days. Meloxicam 0.1 mg/kg was also administered IM SID X 3. Cefazolin 20mg/kg IM TID, was administered for 5 days, when deemed necessary by the Facility Veterinarian.

Post-Operative Procedures: Following surgery, the monkeys were closely monitored during anesthetic recovery for physiological disturbances including cardiovascular/respiratory depression, hypothermia, and excessive bleeding from the surgical sites for 14 days. Supplemental heat was provided as needed. Long term postoperative monitoring included inspection for signs of pain or infection. Daily incision site checks/observations were performed.

Pump Loading and Programming: Monkeys were jacketed with primate jackets (Lomir) that had a pouch to hold the external programmable ambulatory pump (CADD Legacy plus) for timed delivery of isoproterenol through the side access VAPs while the animals were awake. Conditioning to primate infusion jackets began at least two weeks prior to dosing. Under ketamine sedation (0.2 ml/kg of 100 mg/ml ketamine IM) on the morning of test article dosing the prefilled pump was placed into the pump pocket within the jacket and the pump catheter attached to the VAP with the pump programmed to deliver 1mL/minute for 10 minutes. Dead space associated with the pump and VAP catheters were filled with the positive control article (isoproterenol) at the time of loading.

At week 8 post surgery monkeys received a slow (10 min) intravenous (IV) infusion of isoproterenol (0.5 µg/kg/min) at the rate of 1 mL/min.

Results – Catheter Optimization

Mean cardiovascular parameters were calculated for individual monkeys by averaging a total of thirteen 15 second intervals collected over each 6 hour interval from a total acquisition period of 48 hours. Averages of each 6 hour interval monitored were calculated for each parameter and have been displayed in Figs. 3-6 for systemic and left ventricular systolic, diastolic and mean blood pressures as well as heart rate. The duration of entry of personnel into the study room for routine activities was limited and noted in the raw data. The data acquired over that period was not included in the results. This model development effort is an ongoing longitudinal study wherein data acquisition has been continued for the different developmental stages to ensure stability and consistency of data.

The mean systemic systolic, diastolic and mean blood pressure as well as heart rate have been stable and consistent with expected measures in the green monkey.

In the initial trials the mean LV systolic, LV diastolic, mean LVP and heart rate were slightly higher in 3 of the 4 monkeys at later time points compared to baseline, which may have reflected configuration of catheter tips. At 5 mm from the suture groove, the catheter was not long enough to fully extend into the ventricular chamber. The observed delayed hump (double hump) in the LV pressure trace in these monkeys was likely due to the sensor being squeezed by the cardiac wall a second time. A second less likely possibility was that the catheter was contacting the papillary muscles at systole leading to the "double hump" in LVP upon contraction.

Subsequently the trials were repeated with a catheter with a harder tip and also one with a less flexible hard cap for better placement and positioning. But these attempts did not resolve the LVP trace anomaly.

In the final cohort of monkeys receiving a further modified catheter designs more consistent LV pressure measurements have been obtained. This was achieved by using an even longer catheter that permits unrestricted positioning during implantation. In this catheters design iteration catheters with a side oriented sensors versus a top oriented sensor was evaluated. At implantation the side sensor catheter did not immediately provide stable data, however, over time greater wave form consistently was exhibited (Fig.2A & B)

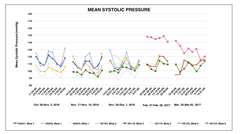

Figure 3A: Change in mean systolic blood pressure over time in the TSE telemetered green monkeys

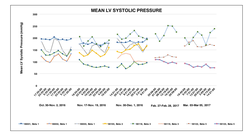

Figure 3B: Change in mean LV systolic blood pressure over time in the TSE telemetered green monkeys

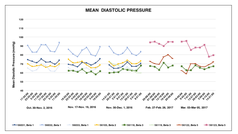

Figure 4A: Change in mean diastolic blood pressure over time in the TSE telemetered green monkeys

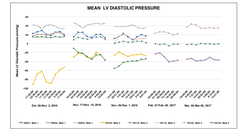

Figure 4B: Change in mean LV diastolic blood pressure over time in the TSE telemetered green monkeys

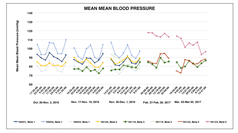

Figure 5A: Change in mean mean blood pressure over time in the TSE telemetered green monkeys

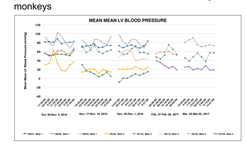

Figure 5B: Change in mean mean LV blood pressure over time in the TSE telemetered green monkeys

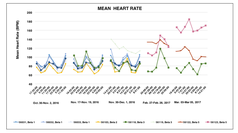

Figure 6A: Change in mean heart rate over time in the TSE telemetered green monkeys

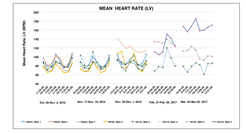

Figure 6B: Change in mean heart rate (LV) over time in the TSE ttelemetered green monkeys

Results – Cardiac Parameter Measurement and Stability

Figures 3A, 4A and 5A demonstrate the systemic systolic, diastolic and mean blood pressure (mmHg) captured over time over the 5 trials. These data have been consistent over time for most monkeys evaluated. The higher values noted for Monkey 56123 could be attributed to the collection being conducted shorty after surgery. The monkey was still recovering from telemeter implantation and additional data will be captured to ensure consistent baseline data.

Heart rate has been consistent in the monkeys over the trial period. Increases in heart rate at immediate post-surgical time points were observed, mirroring surgery associated pressure differences (Fig. 6A). More data will be collected to characterize stability of heart rate data over time.

Figures 3B, 4B and 5B show the respective data from the LV catheter for systolic, diastolic and mean blood pressure (mmHg) captured from the same monkeys over the 5 trials. This data exhibits significant variability, including negative data points in some cases, which is likely attributable to the catheter design limitations in initially telemetered monkeys that contributed to the "double hump" phenomenon noted in the wave forms (Fig. 1). The heart rate, however, exhibited greater consistency across measures (Fig. 6B).

Further data being collected currently in the recently telemetered cohort is expected to demonstrate a cleaner and more dependable dataset from the LV catheter as well.

Results – Isoproterenol Effects on Cardiac Parameters

Figure 7: Change in mean systolic BP over time in the TSE telemetered green monkeys treated with isoproterenol

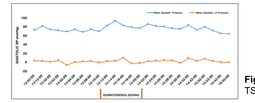

Figure 8: Change in mean diastolic BP over time in the TSE telemetered green monkeys treated with isoproterenol

Figure 9: Change in mean mean BP over time in the TSE telemetered green monkeys treated with isoproterenol

Figure 10: Change in mean heart rate over time in the TSE telemetered green monkeys treated with isoproterenol

Intravenous administration of isoproterenol (ISO) resulted in a transient inotropic and chronotropic response demonstrated by increased LVP and systemic systolic pressure as well as the mean systemic BP possibly independent of any lusitropic effects (Fig. 7 & 9). The minor effect on systemic BP is overshadowed on the graph by the LV pressure data (Fig. 7)

ISO administration did not effect the systemic or the the LV diastolic pressure significantly (Fig.8). The heart rate showed changes following ISO administration (Fig. 10).

Conclusions

- Surgical procedures were established that enabled successful implantation of a novel solid state telemetry transmitter in St. Kitts African green monkeys
- Optimal catheter design for the St. Kitts green monkey was established in order to obtain optimal data from the Stellar TSE telemetry system
- Baseline cardiac activity data, exhibiting parameter stability, continues to be collected using this experimental strategy
- To demonstrate the utility of the TSE system in St. Kitts green monkeys a positive control (isoproterenol) was administered to one monkey, demonstrating a positive inotropic and chronotopic effect
- Data generated with the Stellar TSE's Type PPBTA-XL Telemetry Transmitter over the first year of use have been comparable to other telemetry devices in other nonhuman primate species, supporting the utility of St. Kitts Green Monkeys as an acceptable and reliable alternative test species for safety pharmacology or combination cardiovascular/toxicology studies.