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AFib Protocol Methods

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AFib Protocols



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We use this protocol and it's working

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Abstract

Persistent

atrial fibrillation (AFib) is the most common chronic arrhythmia in adults in the United States and is associated with significant morbidity, including thromboembolic events, stroke, and heart failure. Despite available therapies such as catheter ablation and antiarrhythmic drugs, AFib remains incurable for many patients. Our study aims to develop a large-animal model of AFib in Yucatan miniswine to support investigation of new therapeutic approaches for this disease.

Guidelines

Animal Preparation: Approximately one-year-old Yucatan miniswine (N=6) were enrolled in this study. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) and adhered to ARRIVE guidelines. Animals were housed in a temperature-controlled facility with a 12-hour light-dark cycle. Standard diets and ad libitum water access were provided in the enriched environment to promote well-being. The study adhered to USDA and AWA regulations, and all efforts were made to minimize animal distress.

Before start

Anesthesia and Surgical Approach: Anesthesia was induced using ketamine (11-33mg/kg IM) and/or midazolam (0.1-0.5 mg/kg IM). Isoflurane (1-3%) was administered for maintenance anesthesia for the duration of the procedure. Animals were administered buprenorphine Sr 0.12-0.27 mg/kg for minimizing pain and distress after surgical implantation of the pacing device. Standard aseptic techniques were followed during surgical preparation. The ICM was implanted subcutaneously in the left chest. For pacemaker implantation, the right external jugular vein was accessed using a cutdown with preemptive loose ligation to minimize bleeding. Custom elongated sheaths and stylus shapes were used to position the pacing atrial lead in the right atrial appendage. A Medtronic CRT-P device was implanted subcutaneously in a small pocket in the right chest. Proper lead placement was confirmed with a multi-lead ECG.

Atrial Fibrillation Induction

- 1 ATRIAL FIBRILLATION STUDY: In this aim we will use implanted pace generator with pacing wire to continuously burst or rapid pace the heart to create sustained atrial fibrillation (Afib). Afib will be determined using an implantable cardiac monitor that can be evaluated in conscious animals. Animals will remain in afib for ~3-40 weeks and undergo treatment with a cryopreserved bioabsorbable matrix populated with human neonatal fibroblasts and seeded with Human induced pluripotent stem cell derived cardiac myocytes (hiPSC) implanted on the atrium or no treatment. ~4-16 wks after patch placement if still in AFib a cardioversion procedure will occur. ~4-60 wks after treatment final electrophysiologic testing will be performed. Hemodynamic studies may be performed during any of the above events.
 - ~7-90 days after arrival: Surgery 1 [Pacemaker Implantation and/or ICM placement [see Implantation: Device/Drug Pellet/electrode/material]] will be performed for pace generator and pacing lead, ICM may also be implanted on all animals during the same anesthetic event.
 - ICMs will be monitored 1 to 5 times weekly to confirm pacing stimulation and document atrial fibrillation initiation. The pace generator will be turned off to verify continuous atrial fibrillation and restarted if Afib is not continuous.
 - Long QT Syndrome:
 - 1 to 5 of the swine may be sedated and lightly anesthetized to test for long QT syndrome by pacing protocols to investigate if the syndrome is what is preventing the development of persistent atrial fibrillation. ~30 mins to 1 hour procedure.
 - Rapid Pacing with medication rate control:
 - Beta blockers and calcium channel blockers are not dosed in mg/kg in clinical settings. They are given at standard dosages and increased/decreased based on the clinical response of the patient.
 - Rapid atrial pacing (~400-600bpm) is the stimulus to create atrial fibrillation in a swine model. In some swine, rapid atrial pacing can affect the ventricles depending on how fast the atrial pacing rate gets through the AV node and increases the ventricular rate.
 - To reduce elevated ventricular rate beta-adrenergic blocker Metoprolol Tartrate was tried under veterinary prescription and showed some degree of efficacy of reducing the ventricular rate during intermittent (≤ 5 hrs) rapid pacing. Metoprolol tartrate (short acting)

≤75mg reduced heart rate to safe levels <160 bpm, but with some rapid heart rate (up to ~200bpm) breakthrough.

- We have tested Metoprolol Tartrate up to 150mg without success. We will restart testing at ≤175mg increasing by ≤25mg until the animal demonstrates side effects of lethargy and/or reduced heart rate <50bpm during daytime hours. This will equate to a maximum dose. Heart rates will be confirmed at ~1 hour, ~3.5 hours and ~6 hours after dosing by assessing the internal cardiac monitor (ICM) (loop recorder) and visually at ~30-to-60-minute intervals, per veterinary guidance.
- With the maximum dose set, we will decrease it by ≤25mg, to the Metoprolol Tartrate highest tolerable dose. The highest tolerable dose will be given for ~2 days with rapid pacing tested on day 2, for up to ~5 hours.
- If tolerated on ~day 2, rapid pacing will be tested under the same dosage for ~4 days during working hours. All rapid pacing starts will have direct observation for up to ~1 hour to assess reaction, behavior and ICM-ECG data. Visual checks will be every ~30 minutes, increasing time between visits with stability and per veterinary guidance. ICM rechecks will be at ~2 hour, ~3.5 hours and up to ~5 hours when the rapid pacing is stopped. Heart rate during rapid pacing rates should be <175bpm with an average of ≤150bpm or less, with low arrhythmic events. If any abnormality is noted or heart rate is above range, rapid pacing and dosing will be stopped. If safe, long-acting metoprolol will be tested for ~24-hour pacing.
- If the highest tolerable dose of metoprolol tartrate is safely controlling ventricular response during rapid pacing, long-acting Metoprolol Succinate at double the highest tolerable dose will be started. Behavioral response will be observed for ~2 days, rapid pacing tested on ~day 2. If safe, ~4 days of daytime rapid pacing, described above will be tested. If all is stable after ~4 days, ~24-hour rapid pacing will be started at the beginning of the week, not over a weekend, with the same monitoring events until deemed stable and safe and the duration intensity of observation will be reduced. If any abnormality is noted or heart rate is above, range rapid pacing and dosing will be stopped.

Drug	Dose (mg)	Trial days	Max Days	Total Trials
Metoprolol Tartrate		Tested up to 150mg at this time		
Metoprolol Tartrate	175	2	-----	6 doses/2 days each dose
Metoprolol Tartrate	200	2	-----	
Metoprolol Tartrate	225	2	-----	
Metoprolol Tartrate	250	2	-----	

Metoprolol Tartrate 275 2 -----

Metoprolol Tartrate 300 2 -----

Highest Tolerable Dose 2 Cont. Dose until 4 days of daytime RP + 4 days of rapid pacing

Drug	Dose (mg)	Min Days	Max Days	Total Trials
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Metoprolol Succinate Start at Final Metoprolol Tartrate dose X 2

Metoprolol Succinate 2 Cont. Dose until 4 days of daytime RP + 4 days of rapid pacing

- If Metoprolol Tartrate or Metoprolol Succinate is ineffective, Diltiazem short acting (SA) will be started at $\leq 200\text{mg}$, increased by $\leq 20\text{mg}$ doses until the animal demonstrates side effects of lethargy and/or reduced heart rate $< 50\text{bpm}$ during daytime hours. This will equate to a maximum dose.

- With the maximum dose set, we will decrease it by $\leq 20\text{mg}$, to the Diltiazem SA highest tolerable dose. The highest tolerable dose will be given for ~ 2 days with rapid pacing tested on $\sim \text{day } 2$, for up to ~ 5 hours.

- If tolerated on $\sim \text{day } 2$, rapid pacing will be tested under the same dosage for ~ 4 days during working hours. All rapid pacing starts will have direct observation for up to ~ 1 hour to assess reaction, behavior and ICM-ECG data. Visual checks will be every ~ 30 minutes, increasing time between visits with stability and per veterinary guidance. ICM rechecks will be at ~ 2 hour, ~ 3.5 hours and up to ~ 5 hours when the rapid pacing is stopped. Heart rate during rapid pacing rates should be $< 175\text{bpm}$ with an average of $\leq 150\text{bpm}$ or less, with low arrhythmic events. If any abnormality is noted or heart rate is above range, rapid pacing and dosing will be stopped. If safe, diltiazem extended release (ER) will be tested for ~ 24 -hour pacing.

- If the highest tolerable dose of diltiazem SA is safely controlling ventricular response during rapid pacing, diltiazem ER at double the highest tolerable dose will be started. Behavioral response will be observed for ~ 2 days, rapid pacing tested on $\sim \text{day } 2$. If safe, ~ 4 days of daytime rapid pacing, described above will be tested. If all is stable, ~ 24 -hour rapid pacing will be started at the beginning of the week, not over a weekend, with the same monitoring events until deemed stable and safe and the duration intensity of observation will be reduced. If any abnormality is noted or heart rate is above, range rapid pacing and dosing will be stopped.

Drug	Dose (mg)	Min Days	Max Days	Total Trials
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Diltiazem (short acting) 200 2 ----- 6 doses/2 days each dose

Diltiazem (short acting) 220 2 -----

Diltiazem (short acting) 240 2 -----

Diltiazem (short acting) 260 2 -----

Diltiazem (short acting) 280 2 -----

Diltiazem (short acting) 300 2 -----

Highest Tolerable Dose 2 Cont. Dose until 4 days of daytime RP + 4 days of rapid pacing

Drug	Dose (mg)	Min Days	Max Days	Total Trials
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Diltiazem (Extended Release) Start at final Diltiazem SA X 2

Diltiazem (Extended Release) 2 Cont. Dose until 4 days of daytime RP + 4 days of rapid pacing

- If Diltiazem SA or ER is ineffective, Metoprolol Tartrate + Diltiazem short acting (SA) will be started at ½ highest tolerable dose of each drug from previous tests and is well tolerated, it will be given for ~2 days with rapid pacing tested on ~day 2. If rapid pacing is deemed safe, the dose will be given each morning with daytime only rapid pacing for ~4 days, if this is safely controlling ventricular response, ~24-hour rapid pacing will be started (not over a weekend).

- If the animal demonstrates side effects of lethargy and/or reduced heart rate <50bpm during daytime hours at the combined dosage or is ineffective all dosing will be stopped, the animal will be pulled from the study and euthanized.

- If the drug treatment described above does not control ventricular pacing at a safe, tolerable rate, the animal will be enrolled into a non-recovery procedure and acoustoelectric cardiac imaging (ACI) will be performed for electrical cardiac mapping in pig hearts. Epicardial and endocardial electrocardiogram (ECG) recording may be performed using multiple electrodes and an ultrasound transducer to induce the acoustoelectric effect. This will be done to demonstrate ACI of the cardiac activation wave and compare it to standard multichannel ECG. Controlled pacing of the heart with an electrode may be performed to measure ACI accuracy, resolution, sensitivity, and reproducibility (and quantify their tradeoffs) in detecting a known electrical stimulus. At the completion of the procedure, the pigs will be euthanized by UAC staff without awaking from anesthesia. The heart is harvested and the following post-mortem

activities will be performed:

- Verification of Langendorff system in the pig
- Demonstrate ACI of cardiac activation wave and compare/validate with standard multichannel ECG
- Measure ACI accuracy, resolution, sensitivity, and reproducibility (and quantify their tradeoffs)
- Take tissue punches and perform IHC for characterization of tissue morphology.

Drug	Dose (mg)	Min Days	Max Days	Total Trials
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Metoprolol Tart & Diltiazem SA	1/2 dose Metoprolol Tart + 1/2 dose Diltiazem SA			
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Metoprolol Tart & Diltiazem SA	2	Cont. Dose until 4 days of daytime RP + 4 days of rapid pacing		
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Drug	Dose (mg)	Min Days	Max Days	Total Trials
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Metoprolol Succ & Diltiazem ER	Metoprolol Tart + Diltiazem SA X 2			
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Metoprolol Succ & Diltiazem ER	Cont. Dose until 4 days of daytime RP + 4 days of rapid pacing			
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- ~3-40 weeks after Surgery 1, Surgery 2 Thoracotomy [Matrix-graft placement] [see Implantation: Device/Drug Pellet/electrode/material: lateral thoracotomy] will be attempted on and Matrix/graft placed on treated all animals. If not already implanted, the ICM may be placed or moved at this time. Surgical procedures would occur during the same anesthetic event [see Implantation: Device/drug Pellet/electrode/material surgery: ICM Placement].

- ~4-16 weeks after Surgery 2, Cardioversion (see description above) may be performed if an animal does not revert to sinus rhythm after patch placement. This procedure may be repeated up to 3 times per animal should conversion to sinus rhythm not happen or the animal reverts to atrial fibrillation.

- ~4-208 weeks after Surgery 2, Surgery 3 [Endpoint Study] will occur [see Cardiac: Instrumentation/mapping surgery]. The animal is euthanized while under surgical anesthesia. Heart and organ tissue will be harvested and preserved, and any other tissue if any abnormalities are noted.

- To determine the long-term benefits post matrix graft implantation, we would like to extend the timepoint from ~104 to ~208 weeks between surgery 2 and surgery 3 for



control and treated animals previously in atrial fibrillation and in current sinus rhythm post cardioversion. This is to determine the safety and long-term effects of the matrix graft placement as well as assess degradation of the material.

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