Evaluation of a novel ECG lead placement method in telemetered freely moving cynomolgus monkeys: Assessment of an intravascular biopotential lead

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ABSTRACT

Introduction: ECG is considered as a critical biomarker of cardiac safety pharmacology. ECG signal quality is essential for accurate interval quantification and automated arrhythmia detection.

Methods: We evaluated ECG signal quality over a 6 month period from 15 cynomolgus monkeys with radiotlemetry transmitters using biopotential leads where the negative lead was inserted in the jugular vein and advanced to the superior vena cava (intravascular lead) and the positive lead was placed on the diaphragm at the apex of the heart (diaphragmatic lead). Signal noise and signal-to-noise ratio from this implantation methodology were compared with signals obtained from animals with subcutaneous ECG lead. Macroscopic pathology and histopathology associated with the intravascular lead were evaluated at 6 months post-implantation in six monkeys.

Results: The ECG morphology obtained with the intravascular/diaphragmatic lead placement was comparable to conventional subcutaneous ECG (emulating Lead II) but presented higher amplitudes (P-wave +50.0%; R-wave +30.0%). Signal noise showed a circadian cycle of changes in magnitude for subcutaneous ECG leads that was not observed with this method. The intravascular/diaphragmatic lead placement presented a higher signal-to-noise ratio than subcutaneous ECG leads. No macroscopic abnormality was observed to be associated with the intravascular lead. Mild thickening of the intima/subintima with mild intimal proliferation of the cranial vena cava surrounding the intravascular lead were noted at histopathological examination.

Discussion: The intravascular/diaphragmatic ECG lead placement in cynomolgus monkeys provided reduced signal noise and elevated P-QRS-T amplitudes. The intravascular lead was well tolerated and appeared suitable for chronically instrumented cardiovascular safety pharmacology studies. Further assessments would be warranted to evaluate the potential of this methodology in other species.

Keywords:
Methods
Telemetry
Safety pharmacology
ECG
Cardiovascular
Surgery

1. Introduction

Electrocardiographic (ECG) monitoring in safety pharmacology studies has received considerable attention both from regulators (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2005) and the industry (Pugsley, Authier, & Curtis, 2008). ECG is recognized as a valid biomarker for cardiac safety assessment throughout the drug development process. The challenges associated with automated analysis of ECG tracings obtained from continuous monitoring in freely moving animals have fostered prolific discussions (Chu & Vargas, 2009) and directed the industry into development of computerized tools.

Electromyographic (EMG) activity is generally recognized as the most important source of artifacts to ECGs and can impede automated and manual arrhythmia detection (Authier, Pugsley, Troncy, & Curtis, 2010) or ECG interval quantification. Automated identification of the terminal phase of the T-wave is recognized as a potential source of imprecision during QT measurements (Morganroth, 2001). In this context, the quality of the ECG signal is critical to optimize sensitivity of safety pharmacology assessments.

Several methods have been described for telemetry ECG lead implantation in cynomolgus monkeys. The traditional subcutaneous placement (Authier et al., 2007) is associated with a high level of EMG noise. Intracardiac ECG leads resulted in unstable P–QRS–T morphology (unpublished data), substantial recovery time, high incidence of IC lead malfunction, and high costs (Yao et al., 2009). Epicardial ECG lead placement provides quality ECG with minimal EMG noise (Henriques et al., 2010) but requires a thoracotomy with recovery from an invasive open chest surgery.
The current study evaluated the suitability of an intravascular/diaphragmatic ECG lead configuration in telemetered cynomolgus monkeys for safety pharmacology investigations. Signal quality and long-term ECG lead tolerability were assessed.

2. Materials and methods

2.1. Statement on use and care of animals

During the study, care and use of animals were conducted in accordance with principles outlined in the current Guide to the Care and Use of Experimental Animals published by the Canadian Council on Animal Care and the Guide for the Care and Use of Laboratory Animals published by the Institute of Laboratory Animal Resources. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) before conduct. LAB Research Inc. is an AAALAC accredited facility. All procedures were conducted per Standard Operating Procedures (SOPs).

2.2. Animal housing

The animal room environment was controlled (temperature 21±3 °C, relative humidity 30–70%, 12 h light, 12 h dark, 10–15 air changes per hour) and temperature and relative humidity were monitored continuously. A standard certified commercial primate chow (Certified Primate Diet 2055C™, Harlan Teklad, Madison, WI, USA) was available to each monkey twice daily.

2.3. Animal preparation

Fifteen (15) cynomolgus (Macaca fascicularis) monkeys (ages: 3 to 5 years, wt.: 2.7–3.8 kg) were surgically prepared with telemetry transmitters (prototype TL11M3-D70-PCTR or TL11M3-D70-PCT, DSI, St-Paul, MN, USA) at LAB Research Inc. Surface ECG was obtained from all animals prior to surgery to ensure all animals presented normal cardiac conduction. Prophylactic antibiotics (penicillin 20 000 IU/kg) were administered by intramuscular (IM) injection at least 30 min prior to surgery and once a day for at least 48-hours post surgery. Preemptive analgesia (buprenorphine, Temgesic™, 0.05 mg/kg, Schering-Plough, Welwyn Garden City, Hertfordshire, UK) was administered by IM injection before surgery and every 6 to 12 h for at least three days post-surgery. Animals were placed on a heating pad and inhaled a mixture of oxygen (O2) and isoflurane (AErrane™, Baxter Corporation, Mississauga, ON, CAN) with the O2 flow meter and the vaporizer set at 1.0 L/min, and 2.0%, respectively. Spontaneous breathing was used except for animals presenting pulsatile hemoglobin saturation in O2 (SpO2) lower than 85% in which case, respiratory rate was maintained at 15 breaths/min with an inspiratory airway pressure between 18 and 24 cm H2O using a mechanical ventilator (2002, Hallowell EMC, Pittsfield, MA, USA). During anesthesia, monitoring included heart rate and SpO2 (VetOx 4404™ pulse oximeter, Heska, Fribourg, Switzerland). An abdominal midline skin incision was made followed with an approximately 4 cm longitudinal incision made in the middle of the rectus abdominis muscle (parallel to muscle fibers). The telemetry transmitter was placed on the left side in a pocket between the internal abdominal oblique muscle and the aponeurosis of the transversus abdominis muscle. The negative ECG electrode (Data Science International, St-Paul, MN, USA) was tunneled subcutaneously to a small skin incision at the level of the right internal jugular vein. The vein was isolated and the intravascular biopotential lead was inserted to level in the cranial vein cava cranial to the right atrium. The intravascular biopotential lead was provided ready to use and did not require any alterations prior to insertion in the internal jugular vein. Care was taken to ensure that the intravascular ECG lead was at least 10 mm cranial to the atrium to maintain appropriate P-QRS-T morphology by examining the signal in real time using the telemetry system. The ECG lead insertion site in the jugular vein was selected to be as close as possible to the collar bone to avoid movements of the lead with head and neck movements. Fluoroscopic imaging (Siemens, Siremobil 4U, Germany) was used to confirm anatomical positioning of the intravascular ECG electrode. A small loop (approx. 8 mm diameter) was created with the naked wire from the positive lead and secured with non-absorbable sutures (Novafil™, Polybutester, Syneture, Norwalk, CT, USA) to the diaphragm left of the area contacted by the apex of the heart through a small midline laparotomy distal to the xiphoid process. The position of the diaphragmatic ECG lead was adjusted for individual monkeys at surgical implantation to ensure electrophysiological monitoring parallel to the depolarization axis and to achieve maximal QRS waveform amplitudes. This was achieved by placing the ECG lead at the surface of the skin with simultaneous telemetry monitoring and assessment of the resulting QRS amplitude. Once the optimal position was located, the lead was sutured at the corresponding level on the diaphragm. In all cases, the position of the diaphragmatic lead was located left to the apex of the heart. The arterial pressure catheter was implanted in the right femoral artery and advanced in the aorta as previously described (Authier et al., 2007). Surgical sites were rinsed with warm sterile saline and sutured in anatomical planes. Skin incisions were closed using absorbable buried sutures to reduce the risk of infection from percutaneous suture track, eliminate the need for suture removal and to minimize the risk of self-trauma since there is no protruding material (Sylvestre, Wilson, & Hare, 2002). Data with subcutaneous ECG leads were obtained from animals with comparable telemetry transmitters (TL11M3-D70-PCT, DSI, St-Paul, MN, USA) implanted as described previously (Authier et al., 2007).

Fig. 1. ECG obtained from a conscious cynomolgus monkeys using an intravascular (negative) and a diaphragmatic (positive) biopotential lead. A. Animal 1 at 1 week post-surgery (PR interval 86 ms, QRS 26 ms, QT interval 170 ms, Heart rate 202 beats per min and QTcB 312 ms). B. Animal 2 at 1 month post-surgery (PR interval 118 ms, QRS 34 ms, QT interval 252 ms, Heart rate 112 beats per min and QTcB 344 ms). C. Animal 3 at 6 months post-surgery (PR interval 98 ms, QRS 39 ms, QT interval 250 ms, Heart rate 133 beats per min and QTcB 373 ms).

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2.4. ECG signal acquisition and signal-to-noise ratio

ECG was continuously recorded at 1000 Hz for a period of at least twenty-four (24) hours at 1 week, 1 month and 6 months post-implantation. The ECG morphology was evaluated and interval quantification (PR, QRS, QT, QTcB and RR) was performed. The analysis of the ECG signal-to-noise ratio was performed on twenty-four hour periods from four animals with intravascular biopotential leads and four animals with conventional subcutaneous ECG leads. The analysis was conducted using Ponemah P3 version 5.0 using the P3 Plus analysis module: Electrocardiogram (ECG version 5.1). ECG signals evaluated included: R-H (Height of the R-wave in millivolts from the iso-electric level) and P-H (Height of the P-wave in millivolts from the iso-electric level). The analysis parameter NOISE reported an approximation of the electronic noise level in the ECG cycle. The value reported was the root mean square (RMS) value of the derivative between the two R-wave peaks after excluding the following regions: 10% of the signal following the start R-wave peak, 10% of the signal prior to the end R-wave peak and 10% of the signal around the two largest derivative peaks. If a derivative greater than three times the largest QRS derivative was encountered, T-wave and P-wave regions were not removed. Noise values were converted from (RMS) to ~mV for use in the signal-to-noise results. The mean percent of incomplete complexes with default ECG automated analysis (Ponemah P3 version 5.0, ECG Analysis version 5.10, Data Science International, St-Paul, MN, USA) was calculated every minute from four (4) animals with subcutaneous leads and four (4) animals with jugular and diaphragmatic leads. Incomplete complexes were defined as a complex with missing P-wave, missing T-wave, abnormal P-wave, abnormal T-wave or abnormal QRS.

2.5. Macroscopic pathological assessments and histopathology

Six female animals were humanely euthanized six months after implantation and were subjected to a complete necropsy. The vena cava surrounding the biopotential ECG lead were trimmed and prepared for histopathological examination.

3. Results

3.1. Post-surgical recovery

Recovery from the surgery was comparable to conventional telemetry implantation surgeries. There was no evidence of change in ECG morphology between time points from our evaluation. As expected, modest variations in P–QRS–T amplitude were noted with Table 1

<table>
<thead>
<tr>
<th></th>
<th>R-wave height (mV)</th>
<th>P-wave height (mV)</th>
<th>Noise (~mV)</th>
<th>QRS signal/noise</th>
<th>P-wave height Signal/noise</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subcutaneous ECG leads in cynomolgus monkeys (n=4)</strong></td>
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<tr>
<td>24 hour average</td>
<td>2.63 ± 0.26</td>
<td>0.16 ± 0.04</td>
<td>0.029 ± 0.010</td>
<td>105.9 ± 46.9</td>
<td>6.0 ± 1.3</td>
</tr>
<tr>
<td><strong>Intravascular biopotential and diaphragmatic ECG leads in cynomolgus monkeys (n=4)</strong></td>
<td></td>
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<tr>
<td>24 hour average</td>
<td>3.42 ± 0.31</td>
<td>0.8 ± 0.05</td>
<td>0.013 ± 0.002</td>
<td>279.5 ± 30.1</td>
<td>64.0 ± 9.3</td>
</tr>
</tbody>
</table>

Fig. 2. ECG signal noise (µV) with subcutaneous ECG leads (n = 4) and IV-Diaphragm leads (n = 4) in freely moving cynomolgus monkeys.
respiration but the changes had no significant impact on automated ECG detection.

3.2. ECG signal amplitude and signal-to-noise ratio

The morphology of the ECG complexes recorded at 1 week, 1 month and 6 months was comparable to a subcutaneous ECG approximating a Lead II limb lead configuration (Fig. 1) in all animals, but provided higher amplitudes of the waveform peaks. ECG morphology was comparable between evaluation periods and did not suggest any differences up to six months post-implantation. Subcutaneous ECG lead signal noise increased during day time as with compared with night time while intravascular ECG lead noise level was comparable throughout the day (Fig. 2). Mean signal amplitude and signal-to-noise ratio with intravascular ECG leads were higher than subcutaneous ECG leads (Table 1). The mean P-wave signal-to-noise ratio observed with the intravascular ECG lead was 10.6 times higher than with subcutaneous leads. R-wave signal-to-noise ratio was higher with intravascular ECG leads than subcutaneous ECG leads during day time (Fig. 3). The ECG leads evaluated in the current study provided adequate ECG morphology and limited signal noise over a period of six months. The circadian increase in EMG artifacts observed with subcutaneous leads was not present with intravascular ECG lead which led to increased accuracy of automated ECG detection and reduced signal processing time. ECG intervals from animals with intravascular ECG leads were considered within normal ranges for cynomolgus monkeys (Table 2). At computerized interval detection, the mean percent of incomplete or abnormal complexes with subcutaneous leads was higher than with jugular and diaphragmatic leads (Fig. 4).

3.3. Necropsy and histopathology

As typically observed with telemetry implants, minimal adhesions were noted around the transmitter placement site. No macroscopic abnormalities were observed around the intravascular ECG lead (Fig. 5) and other organs. At histopathological examination, the superior vena cava showed, in order of severity, mild thickening of the intima/subintima with mild intimal proliferation (n = 1) (Fig. 6A and B), mild intimal proliferation (n = 2) or no abnormalities (n = 3). No evidence of inflammation was noted at histological evaluation of the intravascular lead placement site.

4. Discussion

Recent advances in arrhythmia analysis (Cools & Gallacher, 2009) have stimulated the interest for methodologies that minimize ECG signal noise. Quality of interval measurement including QT interval quantification relies on the quality of the waveform (Buckles et al., 2004). The potential for interferences from EMG during ECG evaluations is recognized by regulators in the ICH S7A guideline: “In practice, some effects in the toxic range (e.g., tremors or fasciculation during ECG recording) may confound the interpretation of the results…” (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 2000).

Processing time for arrhythmia detection or interval quantification using automated computerized tools is inversely correlated with the

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**Table 2**

Intravascular biopotential and diaphragmatic ECG leads in cynomolgus monkeys (n = 5).

<table>
<thead>
<tr>
<th></th>
<th>PR-interval (ms)</th>
<th>QRS-interval (ms)</th>
<th>QT-interval (ms)</th>
<th>QTcB* (ms)</th>
<th>RR-interval (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hour average</td>
<td>78.9 ± 3.2</td>
<td>31.1 ± 1.1</td>
<td>202.7 ± 9.2</td>
<td>340.0 ± 7.4</td>
<td>356.4 ± 23.8</td>
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* QT corrected using Bazett’s formula (Bazett, 1920).
quality of the signal waveform. In our experience, non human primates with subcutaneous ECG leads exhibit a higher level of EMG noise than other species commonly used in safety pharmacology such as canines (Chaves et al., 2007) or minipigs (Stubhan et al., 2008). Based on the results from the current study, the intravascular ECG leads yielded high amplitude P–QRS–T with improved signal to noise ratio in cynomolgus monkeys when compared with subcutaneous ECG leads. Modest variations in ventricular depolarization axis were observed between animals in the current study but were not quantified. Calculation of the ventricular depolarization axis in each animal from the surface ECG recorded in conscious and standing position prior to the surgery could facilitate ECG lead placement with this methodology by providing guidance on the angle to select for the diaphragmatic lead placement relative to the intravascular (jugular) lead.

Fluoroscopic imaging was used to assess anatomical placement of the intravascular lead. Use of fluoroscopic imaging was not considered essential in subsequent animals prepared with the same methodology since the catheterization of the cranial vena cava from a jugular vein was easily achieved following natural vascular anatomy. Additionally, simultaneous ECG monitoring by telemetry during surgery was considered critical to properly position the intravascular lead. The most stable ECG morphology was obtained with the intravascular lead inserted in the cranial vena cava sufficiently above the atrium to minimize the impact of heart movements in the mediastinum in the freely moving state. Biocompatibility observed in the current study was comparable to previous in-vivo results with the components of the intravascular lead; silicon (Melvin, Fissell, Roy, & Brown, 2010) and uncoated stainless steel (Mikhalovska et al., 2011).

The upper thorax in cynomolgus monkeys is relatively stable and most movements (e.g., feeding) are predominantly undertaken using both arms while the head of the animal is focused on visual monitoring of the environment. The effect of movements on stability of the ECG morphology could be evaluated in species naturally presenting more important head and neck mobility such as canines.

In conclusion, the intravascular and diaphragmatic ECG leads provided suitable results for telemetry monitoring in cynomolgus monkeys. The high P–QRS–T amplitude, the enhanced signal-to-noise ratio and the post-operative recovery were considered as positive features of this surgical method compared with other available approaches.
Fig. 6. A. Vena cava (hematoxylin and eosin, 20X) with thickening of the intima/subintima with intimal proliferation. B. Vena cava (hematoxylin and eosin, 400X) with thickening of the intima/subintima with intimal proliferation.

References


