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Effects of group housing on ECG assessment in conscious cynomolgus monkeys

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ABSTRACT

Introduction: Assessing the cardiovascular safety of new chemical or biological entities is important during pre-clinical development. Electrocardiogram (ECG) assessments in non-human primate (NHP) toxicology studies are often made using non-invasive telemetry systems. We investigated whether ECG recording was feasible during group housing of NHPs, rather than the usual single housed arrangement, and whether it would impact the data collected or affect the ability to detect drug-induced changes in QTc interval.
Methods: Following a period of acclimatisation to jackets, cynomolgus monkeys (3 males and 3 females) were housed in same sex groups of 3. Female monkeys were administered 4 doses of vehicle while male monkeys were administered vehicle, 15, 45, and 135 mg/kg moxifloxacin. Each dose was administered on a separate dosing day. The same dosing protocol was repeated with the animals singly housed and the results from the two phases were compared including assessment of statistical power.
Results: Heart rate (HR) was significantly lower, and PR and QT intervals were significantly higher, at multiple time points when the animals were group housed compared with the singly housed phase. QRS duration and QTc interval were less affected. Moxifloxacin increased QT and QTc intervals but had no consistent effect on HR, QRS duration or PR interval under group housed or singly housed conditions. Power analysis suggested that group housing did not adversely affect the magnitude of detectable changes of ECG parameters. In general, detection of slightly smaller changes was achieved under conditions of group housing.
Discussion: The current study shows group housing to be technically possible during non-invasive ECG recording, resulting in lower resting heart rates and small improvements in sensitivity of detection of drug-induced effects. Given the psychological benefits of group housing for NHPs, it is a refinement that should be considered when conducting ECG assessments in NHP toxicology studies.

1. Introduction

Adverse effects on the cardiovascular system are a main cause of attrition in the development of new pharmaceuticals (Laverty et al., 2011). An assessment of the cardiovascular effects of a new chemical or biological entity is, therefore, of paramount importance during the pre-clinical risk assessment. Traditionally, cardiovascular safety assessments are performed in stand-alone safety pharmacology studies using implantable telemetry systems (Leishman et al., 2012). Recently non-invasive telemetry systems have become more widespread in usage. These systems are particularly appropriate for incorporation into regulatory toxicology studies providing continuous electrocardiogram (ECG) assessment in unrestrained animals (Redfern et al., 2013; Vargas, Amouzadeh, & Engwall, 2013), which represents an improvement on the traditional snapshot methods of ECG collection usually performed under restraint. Use of such improved techniques is especially important for non-clinical packages where no stand-alone cardiovascular safety pharmacology study is performed and the cardiovascular assessment is incorporated into the 1-month toxicology study, an option for biopharmaceuticals (ICH Harmonised Tripartite Guideline, 2011) or new pharmaceuticals or biopharmaceuticals intended for

Abbreviations: AAALAC, Association for Assessment and Accreditation of Laboratory Animal Care; ANCOVA, analysis of covariance; ANOVA, analysis of variance; CDSER, Center for Drug Safety Evaluation and Research; CI, confidence interval; DSI, Data Sciences International; ECG, electrocardiogram; HR, heart rate; ICH, International Conference on Harmonisation; JET, jacketed external telemetry; NHP, non-human primates; SIMM, Shanghai Institute of Materia Medica.

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life-threatening malignancies (ICH Harmonised Tripartite Guideline, 2009).

An additional benefit of non-invasive telemetry systems is that telemetry signals can be transmitted on multiple frequencies making group-housing of animals a possibility. Due to technical limitations with some traditional implantable telemetry systems, animals that are normally group housed have to be separated and singly housed during recording periods (usually of 24 h duration). Few studies have investigated the impact of housing arrangement on cardiovascular data recorded from laboratory animals. However, in one study in dogs, in which single and group housing were compared, housing configuration was shown to impact both absolute values and variability of heart rate (HR) and blood pressure, with pair housing appearing to offer the best haemodynamic stability (Klumpp, Trautmann, Markert, & Guth, 2006). Non-human primates (NHPs) exist in groups in nature and social interaction is known to be required for psychological well-being (DiVincenti & Wyatt, 2011). Indeed, several organisations involved in animal research in NHPs (e.g. the Association for Assessment and Accreditation of Laboratory Animal Care [AAALAC], the International Primatological Society, and the Institute for Lab Animal Research) have stated that group or social housing of NHPs should be considered the default arrangement. When housed in social groups, NHPs are able to cope more effectively with potential stressors, to exhibit less stereotypical behaviour and have a more balanced temperament (DiVincenti & Wyatt, 2011).

The current study was performed during the validation of the Data Sciences International (DSI) jacketed external telemetry (JET) system at Shanghai Institute of Materia Medica (SIMM). The aim was to investigate whether group housing of cynomolgus monkeys during non-invasive ECG recording was feasible and whether it would impact on the data collected compared with single housing. In particular, we investigated whether housing conditions affected the ability to detect drug-induced changes in ECG. In these studies, ECG was recorded alone without any blood pressure measurements.

2. Methods

2.1. Animals and housing

The study was performed at an AAALAC accredited facility. All animal procedures were performed in compliance with the animal welfare policies and guidelines of SIMM, Chinese Academy of Science. The study protocols were reviewed and approved by the Institutional Animal Care and Use Committee.

Seven cynomolgus monkeys (3 males and 4 females) (*Macaca fascicularis*) (Guangxi Weimei Bio-tech Co. Ltd., Guangxi, China and Guangdong Landau Biotechnology Co. Ltd., Guangzhou, China) aged 3–5 years, weight 3.70–6.39 kg were used on the study. Animals were drug-naïve having not been used on a study previously. The study was conducted in a single animal room with males and females on opposite sides of the room. Animals were normally housed in single sex groups of 3 except during the second phase of the study when single housing was required. In both group and single housed phases the pens used measured approximately 2 m × 1.5 m × 2 m.

Animals were offered a set weight of diet (PMI Nutrition International, LLC) once each dosing day in the afternoon (approximately 4 h after dosing) and twice daily during dose-free periods, in the morning and afternoon. The animals were also given fruit, vegetable, or additional supplements as a form of environmental enrichment. Toys (e.g. platforms, treats etc) were also provided. Animals had free access to tap water and samples were analysed every 6 months for specified microorganisms and environmental contaminants. Bedding was provided (Suzhou Zhulin Trade Co. Ltd.) and the animal room temperature and relative humidity monitored and maintained at 20 °C to 26 °C and 40% to 70%, respectively. Rooms were illuminated using a 12 h natural and artificial light/12 h dark cycle. The lights were gradually switched on at 7 am and gradually switched off at 7 pm.

2.2. Acclimatisation

Animals were acclimatised to the jacketing procedure during a period of 2–3 weeks prior to the first dosing phase. Conscious animals were jacketed whilst restrained in chairs. On the first jacketing occasion anaesthesia was used (ketamine 2 mg/kg i.m.), but this was found to be unnecessary on subsequent occasions. During week 1 of the acclimatisation, the duration of jacketing started at 15 min with the animal remaining in the chair and this was increased to 1 h and 2 h with the animals being returned to the home pen in groups. During week 2, the length of time in the jacket was gradually increased to 24 h.

2.3. ECG recording

On the last day of acclimatisation, and on all subsequent dosing days, ECGs were recorded from the animals using the JET system (Data Science International, USA). During restraint, standard ECG skin electrodes and a body temperature probe were attached to the animals with the ECG leads in the following configuration: negative lead on the right side of the upper chest, positive lead on the left side of the lower chest (for Lead II), positive lead on the left side of the upper chest (for Lead I), reference lead on the right side of the lower chest. The body temperature probe was positioned on the left side of the upper chest. Local hair removal on the areas of skin electrode attachment had been previously performed. Undershirts and jackets were placed on the animals and the electrodes connected to the JET device (JET-3ETA-BP), which was placed in the rear pocket of the jacket. The animals were returned to their pens and data were transmitted from each JET device to the data acquisition PC via Bluetooth® receivers positioned within the animal unit. Data were acquired using Ponemah v5.0 software and the ECG signals were sampled at 750 Hz. On dosing days, data were collected for approximately 1 h prior to dosing until 24 h after dosing.

2.4. Dosing and housing

In phase 1, 3 female (Group 1) and 3 male (Group 2) animals were housed for the duration of the phase in same sex groups (3 monkeys/pen). Group 1 animals were administered vehicle on days 1, 4, 9, and 16 and Group 2 animals were administered vehicle, 15, 45, and 135 mg/kg moxifloxacin on days 1, 4, 9, and 16, respectively. Doses were administered orally, by nasogastric intubation, in a dose volume of 5 ml/kg. The vehicle used was water containing 0.5% w/v hydroxypropyl methylcellulose (Methocel E4M)/0.1% w/v Polysorbate 80.

In phase 2, 3 female (Group 1) and 3 male (Group 2) animals were housed singly (1 monkey/pen) on dosing days and returned to same sex groups on non-dosing days after completion of the ECG recording. Despite the single housing on dosing days, some contact was still possible between monkeys in adjacent cages. Group 1 animals were administered vehicle on days 1, 4, 15, and 23 and Group 2 animals were administered vehicle, 15, 45, and 135 mg/kg moxifloxacin on days 1, 4, 15, and 23, respectively. All 3 males and 2 of the female monkeys used in phase 1 were also used in phase 2; however, one of the female monkeys from phase 1 had to be replaced due to a soft tissue mass developing on the thorax. This was thought to be unrelated to the jacketing process. The replacement animal received acclimatisation to the jacketing procedure prior to use on phase 2.

A period of 3 months elapsed between the end of phase 1 and the start of phase 2. Jacket training was performed on 5 occasions at regular intervals throughout this period. Inspection of the heart rate profiles obtained from a training session just prior to the start of phase 2 showed that the animals remained acclimatised to the jacketing procedure.

In both phases a blood sample (approximately 1 ml) was taken from a cephalic vein at 4 h post-dose for proof of drug exposure. Samples

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