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Original article

A cardiovascular monitoring system used in conscious cynomolgus monkeys for regulatory safety pharmacology: Part 2: Pharmacological validation

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Abstract

Introduction: This project addresses the validation study design of a test system using a telemetered non-human primate model for cardiovascular safety pharmacology evaluation. **Methods:** In addition to non-pharmacological validation including installation and operation qualifications, performance qualification (locomotor activity and cardiovascular evaluations) was completed on free-moving cynomolgus monkeys by quantifying the degree of cardiovascular response measured by the telemetric device to various positive control drugs following their intravenous administration. Remifentanil (0.0005, 0.001, 0.002, 0.004, 0.008 and 0.016 mg/kg) was given to induce bradycardia and hypotension. Medetomidine (0.04 mg/kg) was used to induce an initial phase of hypertension followed by hypotension and bradycardia. Esmolol (0.5, 1.0 and 2.0 mg/kg) was used to induce bradycardia. Dopamine (0.002, 0.008, 0.01, 0.02, 0.03 and 0.05 mg/kg/min) was infused over 30 min to induce an increase in arterial and pulse pressures and tachycardia. Amiodarone (0.4, 0.8 and 1.6 mg/kg/min) was infused over 10 min to induce QT interval prolongation. Potassium chloride (0.08 mEq/kg/min) was infused for periods of less than 30 min to induce electrocardiographic (EKG) changes characteristic of hyperkalemia. Reliability was evaluated over 60 days. **Results:** Monitoring with a reference methodology and the telemetry system was important in order to evaluate precision and accuracy of the test system. Positive control drugs produced a wide range of cardiovascular effects with different amplitudes, which were useful in identification of the limits of the test system. **Discussion:** Reference monitoring methods and selection of a battery of positive control drugs are important to ensure proper test system validation. Drugs inducing not only QT prolongation but also positive and negative chronotropic effects, positive and negative systemic arterial pressure changes and ECG morphology alterations were useful to identify test system limitations during performance qualification. ECG data processing at significantly elevated heart rates revealed that a trained observer should review all cardiac cycles evaluated by computer.

Keywords: Cardiovascular; Methods; Non-human primates; Pharmacology; Preclinical; Safety

1. Introduction

Many studies using the cynomolgus monkey (*Macaca fascicularis*) are non-clinical drug trials involving safety profiling, as promulgated by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (US FDA — ICH S7A, 2001; US FDA — ICH S7B, 2004), and particularly specific safety pharmacology evaluations. Studies conducted under the guidance of the ICH are designed to discover and characterize the potential adverse cardiovascular effects of biologically active new chemical entities (NCE) that may present as an unintended consequence of NCE administrations (ICH S7A, ICH S7B). Monkeys are one of the most commonly used preclinical safety species, after dogs, because of their genetic,

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cardiovascular and metabolic similarities to humans, but only a few published reports illustrate the usefulness of telemetry in conscious monkeys (Benardeau, Weissenburger, Hondeghem, & Ertel, 2000; Gauvin, Tilley, Smith, & Baird, 2006; Hassimoto & Harada, 2003; Kaufman & Detweiler, 1999). A recent study (Gauvin et al., 2006) has published a significant dataset on normal values for core body temperature, hemodynamic, and ECG parameters for well-acclimated, freely moving laboratory-housed cynomolgus monkeys using radiotelemetry recording methodologies. This study also highlighted the numerous advantages of using telemetered cardiovascular safety pharmacology devices, such as obtaining physiological measurements from awake and freely moving laboratory animals without introducing either physical or chemical mediated stress or restraint artefacts in the data. Moreover, there has been explicit acknowledgement in ICH and other regional regulatory drug safety testing guidelines that conditions present during routine telemetric monitoring may most closely approximate the normal physiological state of the animal, and therefore safety endpoints evaluated under such conditions may consequently demonstrate the greatest predictive validity to outcomes of similar testing in humans. Indeed, the authors of this recent publication presented a convincing list of advantages for using remote radio-monitoring in preclinical safety pharmacology. This list of advantages includes a humane, affordable, accurate, readable (without human contact, and as such eliminating a major source of interfering stress), reliable and, for long-term use, without special animal care or maintenance method (Gauvin et al., 2006). Moreover, another recent study tested the rhesus monkey telemetry model as a preclinical predictor of pro-arrhythmic potential (specifically QT interval prolongation) of human pharmaceuticals (Chaves et al., 2006). Limited information is available on precise analysis of the ECG (Hassimoto & Harada, 2003; Horii et al., 2002; Ohmura et al., 1999). Particularly, the measurement of corrected QT interval (QTc by Bazett (1920) and/or Fridericia's (1920) correction formula) in cynomolgus monkey appeared to be a useful approach to evaluate the potential cardiotoxicity of histamine H_1 receptor antagonists (Horii et al., 2002; Ohmura et al., 1999). Nicardipine (a Ca^{2+} channel blocker) at 30 mg/kg (po) caused sustained hypotension and tachycardia (Horii et al., 2002). Results obtained with various agents have also confirmed the sensitivity of the non-human primate model for non-clinical assessments of the potential for drug-induced QT prolongation (Ando et al., 2005). These recent studies provided convincing arguments showing that these procedures represent the contemporary industry's preferred practices for measuring such cardiovascular parameters under the ICH guidelines, and are amenable to routine use in a variety of other relevant safety/efficacy studies. They also confirmed, in our opinion, the necessity to validate the telemetric device with *ex vivo* and *in vivo* non-pharmacological and pharmacological evaluation of precision, accuracy and repeatability.

Even if non-human primates are routinely used for regulatory cardiovascular safety pharmacology, validation study results are rarely reported in scientific literature, and available data is limited. The aim of the current project is to evaluate, similarly to what was done with rodents, the installation, operation and performance qualification of a telemetry system using a telemetered non-human primate model.

2. Methods

2.1. Hardware and software

This study evaluated the following components of the Data Science International (DSI, St-Paul, MN, USA) telemetric system:

- Temperature and physical activity transmitters (Model TA10TAD70)
- Pressure, biopotential, temperature and physical activity monitoring transmitters (Model D70-PCT)
- Telemetry receivers (Model RMC-1)
- Telemetry Data Exchange Matrix (Data Exchange Matrix™)
- Ambient Pressure Reference (Model APR1)
- Data acquisition and analysis software (Dataquest A.R.T. ™ Gold Version 3.01)
- Electrocardiogram analysis software (Physiostat™ ECG Analysis 4.01)

The system was installed by the DSI technical staff on a desktop computer (Optiplex GX270™, Dell, North York, ON, Canada). The study was conducted in accordance with the Good Laboratory Practice (GLP) regulations of the United States Food and Drug Administration (21 CFR Part 58 and subsequent amendments). The test plan consisted of the four phases presented below. The serial number of each system components was recorded, verified at each step of validation, and included in the study report.

2.2. In-vivo evaluation

During the study, the care and use of animals were conducted in accordance with the principles outlined in the current Guide for the Care and Use of Laboratory Animals, a National Research Council publication (Anonymous, 1996). LAB Research Inc.'s facility is AAALAC accredited.

Four cynomolgus monkeys (*M. fascicularis*), 2 males and 2 females, were used for ECG, systemic arterial blood pressure (SAP) and locomotor activity evaluations. Surgical implantation and anesthesia were performed as described in Part 1 of the current article (Authier et al., 2007). At study initiation, monkeys were 2.7 to 5.8 years old and weighed between 2.5 and 4.4 kg. The animal room environment was controlled (temperature 21 ± 3 °C, humidity 30–70%, 12 h light, 12 h dark, 10-15 air changes per h) 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, except on the day prior to surgery at which time animals were fasted overnight.

2.2.1. Procedures with drug-induced cardiovascular effects

Six drugs with known cardiovascular effects were administered to 4 telemetered cynomolgus monkeys. This included drugs

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Table 1
Cardiovascular positive control drugs

IV bolus	Dose level (mg/kg)
Remifentanil	0.0005
	0.001
	0.002
	0.004
	0.008
	0.016
Medetomidine	0.04
Esmolol	0.5
	1.0
	2.0
IV infusions (duration)	**Dose rate**
Dopamine mg/kg/min (30 min step-infusion)	0.002
	0.008
	0.01
	0.02
	0.03
	0.05
Amiodarone mg/kg/min (10 min)	0.4
	0.8
	1.6
Potassium chloride mEq/kg/min (up to 30 min)	0.08

mainly affecting HR (either decreasing it, remifentanil; medetomidine; and esmolol; or increasing it, dopamine), SAP (either decreasing it, remifentanil; and esmolol; or increasing it, dopamine; or with a biphasic effect, medetomidine) or ECG [QT, QTcV (van de Water), QTcF (Fridericia), QTcB (Bazett), amiodarone; and multiple effects on ECG, potassium chloride]. Most drugs including remifentanil (Ultiva®, Abbott Laboratories Ltd., Vaughan, ON, Canada), naloxone (Sabex, Boucherville, QC, Canada), dopamine (Inotropin™, Bristol-Myers Squibb, Montreal, QC, Canada), esmolol (Brevibloc®, Baxter Corporation, Mississauga, ON, Canada) and amiodarone (Sabex, Boucherville, QC, Canada) were purchased from a local pharmacist (Pierre Dannel, Laval, QC, Canada). Potassium chloride (Hospira Healthcare Corporation, St-Laurent, QC, Canada) and medetomidine (Domitor®, Novartis, Missisauga, ON, Canada) were purchased from a veterinary product distributor (CDMV Inc., St-Hyacinthe, QC, Canada). To minimize artifacts following IV administration, drugs available as commercial solution forms except for remifentanil that needed to be reconstituted, were given to unrestrained animals equipped with a jacket and tether connected to a continuous infusion line and a pump (Table 1). These drugs were mainly selected with regards to their very short elimination half-life (esmolol, remifentanil), and/or the possibility to effectively antagonize their effects (remifentanil, medetomidine, potassium chloride).

2.3. Statistical methods

Drug-induced effects were evaluated using a two-way ANOVA for repeated measures (SAS, Cary, North Carolina, USA). *A posteriori* contrasts were conducted using Dunnett's test. To facilitate analysis, a period of data collection was selected with regards to observed peak effect following drug administration and compared to a similar data collection period prior to drug administration. For example, short-acting cardiovascular effects were observed immediately after remifentanil injection. As a result, statistical analysis was conducted on a 1 min period average starting 1 min after administration, which was compared with the average from 1 min prior to remifentanil administration. Statistical tests were performed at the 0.05 threshold of significance. Mean ± SD data are presented.

2.4. Acceptance criteria

Identification of hemodynamic and ECG changes following administration of selected drugs with known effects was considered to be an important component of performance qualification of the system. A validation study should be performed over a period of time permitting assessment of the system reliability and consistent intended performance. As required by 21 CFR Part 11, reliability and consistent intended performance were evaluated over a 60-day period, which is considered representative of normal conditions of use for the system.

3. Results

3.1. In-vivo evaluation

3.1.1. Drug-induced cardiovascular effects

3.1.1.1. Remifentanil. The telemetry system identified HR and diastolic SAP modifications ($p<0.05$) with the greatest effects observed at 0.016 mg/kg ($p<0.05$) (Table 2). An increased QT interval was identified after the administration of 0.016 mg/kg ($p<0.05$), but QTcV, QTcF and QTcB were not statistically different.

A dose of 0.016 mg/kg produced evidence of electromechanical dissociation in 1 animal. This observation was preceded by sino-atrial node blockade. Naloxone was given as an IV bolus (0.1 mg/kg) to reverse the effect of remifentanil. Upon return to sinusal rhythm, increased T-wave amplitude (Fig. 1) and ventricular premature contractions (Fig. 2) were noted for this animal.

Table 2
Cardiovascular effects of remifentanil in conscious cynomolgus monkeys ($n=4$)

Dose level (mg/kg)	Systolic SAP (mmHg)	Mean SAP (mmHg)	Diastolic SAP* (mmHg)	Arterial pulse pressure (mmHg)	HR* (beats/min)
0	130±18	111±16	90±13	40±5	159±25
0.0005	131±15	111±14	89±11	43±5	149±39
0.001	133±10	113±8	93±6	40±5	160±31
0.002	137±9	116±9	94±8	44±3	191±20
0.004	135±9	112±8	89±9	46±6	192±20
0.008	130±21	102±16	78±16	52±13	151±56
0.016	110±50	80±41	63±32†	47±19	106±62†

*$p<0.05$ ANOVA; †$p<0.05$ Dunnett's test compared to baseline, analysis of data recorded over 1 min starting 1 min after administration.

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3.1.1.2. Medetomidine. A biphasic effect of medetomidine on SAP was identified by the telemetry system on all dosing occasions (Table 3). As expected, systolic, diastolic and mean SAPs increased, followed by a hypotensive phase associated with a decrease in HR. A statistically significant increase in QT, QTcV and QTcF intervals was noted at 30 min compared to baseline, with some episodic occurrence (noted two times, in one monkey) of sinus arrhythmia (second degree atrioventricular block — Mobitz II) during the first 30 min after injection.

3.1.1.3. Esmolol. A significant decrease in systolic, mean and diastolic SAPs combined with a decrease in pulse pressure and HR was identified with the telemetry system. The highest dose (2.0 mg/kg) induced the greatest changes (Table 4), particularly on systolic and mean SAPs.

3.1.1.4. Dopamine. At lower doses (0.002, 0.008 and 0.01 mg/kg/min), dopamine infusion had no apparent effect on cardiovascular monitoring parameters. With a 0.02 mg/kg/min infusion rate, the diastolic SAP showed a decrease without reaching statistical significance but translated in a significant increase in arterial pulse pressure (Table 5). This effect on arterial pulse pressure was also present with an infusion rate of 0.03 mg/kg/min, but it was associated with a return to baseline value for diastolic SAP and an increase in systolic SAP that did not reach statistical significance. With an infusion rate of 0.05 mg/kg/min, the increase in systolic SAP was statistically significant as was the increase in arterial pulse pressure, but the latter showed a statistically lower increase compared to the one observed with the two previous infusion dose rates.

3.1.1.5. Amiodarone. Following administration of 4, 8 and 16 mg/kg (total dose), prolongation of the QT ($p<0.0001$), QTcV ($p<0.0005$), QTcF ($p<0.005$) and QTcB ($p<0.05$) intervals were noted for all animals. QT prolongation observed at 4 (Fig. 3) and 8 mg/kg were equally significant, with a maximal 21% increase in QT duration compared to saline. Administration of the highest dose (16 mg/kg) induced an increase in QRS duration (+32%), with an increase in QT (+26.1%) and QTcB (+11%).

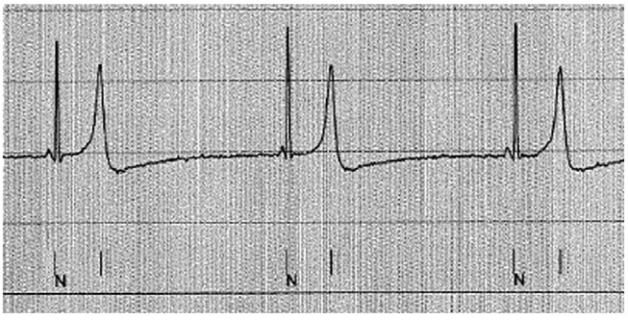

Fig. 1. Increased T wave amplitude after myocardial ischemia in a male cynomolgus monkey. The ECG was obtained shortly after electromechanical dissociation caused by remifentanil at high dose (0.016 mg/kg) and reversed by naloxone (Derivation II). The T wave amplitude was increased for a period approximately 2 min starting 30 s after return to sinusal rhythm with ventricular contractions.

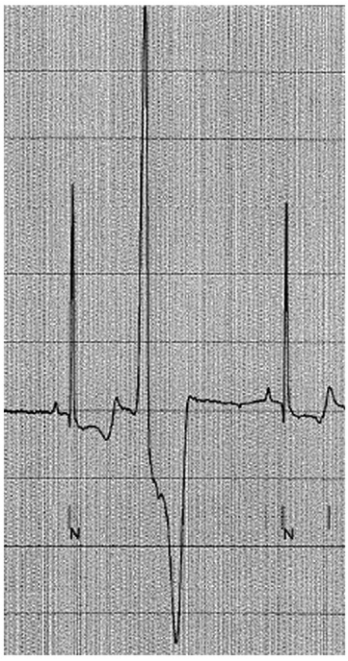

Fig. 2. Premature ventricular contraction (PVC) observed in a male cynomolgus monkey after electromechanical dissociation subsequent to remifentanil at high dose (0.016 mg/kg). A total of ten (10) PVC were observed during a period of 1 min shortly after reversal of remifentanil with naloxone (Derivation II).

3.1.1.6. Potassium chloride. In the first monkey (infusion over a 2 h and 6 min period) the ECG showed T-wave peak, widening and slurring of the QRS complex, flattening and loss of P-wave and atrial fibrillation. Early signs of hyperkalemia are presented in Fig. 4. Emergency treatments were provided to the animal. Subsequent infusions were limited to 30 min, before all these ECG changes occurred for the other animals to minimize risks of serious deleterious effects. They were indeed stopped at the first occurrence of ECG signs of hyperkalemia: an increase in T-wave amplitude ($p<0.05$) combined with a decrease in R-wave amplitude ($p<0.05$) were noted for all animals.

Table 3
Cardiovascular effects of medetomidine in conscious cynomolgus monkeys ($n=4$)

SAP (mmHg)	Time post-Rx (min)		
	Baseline	1	30
Systolic*	118±13	153±12†	96±8
Mean*	100±12	129±12†	83±6†
Diastolic*	84±15	109±28†	69±28
HR (bpm)*	143±39	125±33	95±17†
ECG intervals (s)			
PR	0.103±0.008	0.099±0.018	0.109±0.014
QRS	0.306±0.008	0.305±0.008	0.320±0.010
QT*	0.206±0.048	0.211±0.031	0.275±0.031†
QTcV (van de Water)*	0.249±0.039	0.253±0.022	0.302±0.021†
QTcF (Fridericia)*	0.269±0.033	0.265±0.024	0.317±0.025†
QTcB (Bazett)	0.308±0.026	0.297±0.026	0.341±0.028
RR*	0.445±0.161	0.517±0.131	0.684±0.206†

*$p<0.05$ ANOVA; †$p<0.05$ Dunnett's test compared to baseline; †$p<0.01$ Dunnett's test compared to baseline, analysis of data recorded over 1 min at specified time.

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4. Discussion

Procedures with pharmacological manipulations are critical to the validation protocol most importantly for performance qualification. Arterial pressure can be divided into steady (mean SAP) and pulsatile components (pulse pressure) (Berne & Levy, 1992). Mean SAP is determined by cardiac output and vascular resistance. The pulse pressure component, representing the variation in pressure around the mean, is influenced by left ventricular ejection, large artery stiffness, early pulse wave reflection, and HR. Cardiac output, peripheral vascular resistance and artery stiffness influence systolic SAP. In contrast, diastolic SAP is determined mainly by peripheral vascular resistance and cardiac output.

The predominant and usual effect of opioids on HR is to produce bradycardia resulting from stimulation of the central vagal nucleus. Remifentanil is an ultra short-acting (μ)-opioid agonist that induces bradycardia (Elliott et al., 2000) and hypotension in humans (Schuttler et al., 1997). Conversely, remifentanil IV bolus (0.002 mg/kg) administered to human volunteers resulted in a transient increase in SAP and HR (Glass et al., 1993). Similar dual biphasic changes (increase followed by decrease in tension) with increasing concentrations of fentanyl were reported in basal canine epicardial coronary artery rings (Introna, Bridges, Yodlowski, Grover, & Pruett, 1995). Although increases in SAP and HR were not significant at similar doses in monkeys (0.002 mg/kg), hypotension was accurately identified at higher doses (Table 2). In dogs, remifentanil produces hemodynamic effects, which include decreases in contractility, cardiac output, HR and SAP (James et al., 1992). In our study, a more pronounced effect was observed on HR and SAP at the highest dose tested (*i.e.* 0.016 mg/kg). The fact that the effect was more pronounced on diastolic SAP than on systolic and mean SAPs suggests a possible decrease in peripheral vascular resistance associated to negative inotropic and chronotropic effects. Indeed, pharmacological studies evaluating alfentanil, fentanyl, and sufentanil in the dog demonstrated direct peripheral vessel smooth muscle relaxation (White, Reitan, Kien, & Thorup, 1990). Direct effects of remifentanil on smooth muscles were also shown on isolated rat tissues (Unlugenc et al., 2003). Based on our data, the same response may be present in cynomolgus monkey. A decrease in HR induces QT interval (Bazett, 1920) physiological prolongation, which was identified by the system after administration of remifentanil at 0.016 mg/kg. As expected, no statistically significant differences were present for QTcV, QTcF, and QTcB. Indeed, opioids may depress cardiac conduction (Fattorini et al., 2003). Other studies have reported that fentanyl slowed atrioventricular node conduction, and prolonged RR interval, atrioventricular node refractory period and Purkinje fiber action potential duration (Blair, Pruett, Introna, Adams, & Blaser, 1989; Royster, Keeler, Haisty, Johnston, & Prough, 1988). Opioids can also prolong the QT interval (Blair, Pruett, Crumrine, & Balser, 1987), but the overall effect of opioid anesthesia is anti-arrhythmic (Atlee & Bosnjak, 1990). We observed cardiac brady-arrhythmia and ECG signs of myocardial ischemic suffering, with ventricular premature contractions and increased T-wave amplitude (Somers, Brady, Perron, & Mattu, 2002), in one animal after remifentanil injection at the dose of 0.016 mg/kg.

Table 4
Cardiovascular effects of esmolol in conscious cynomolgus monkeys ($n=4$)

Dose level (mg/kg)	Systolic SAP* (mmHg)	Mean SAP* (mmHg)	Diastolic SAP* (mmHg)	Arterial pulse pressure* (mmHg)	HR* (beats/min)
0	139±11	121±10	98±9	43±4	194±36
0.5	131±9†	112±8†	92±6	40±4†	172±34
1.0	132±9	113±7	94±5	37±5	168±31
2.0	124±7†	107±5†	87±5†	37±4†	167±17†

*$p<0.05$ ANOVA; †$p<0.05$ Dunnett's test compared to baseline; †$p<0.01$ Dunnett's test compared to baseline, analysis of data recorded over 1 min immediately following administration.

Table 5
Cardiovascular effects of dopamine infusion in conscious cynomolgus monkeys ($n=4$)

Dose level (mg/kg/min)	Systolic SAP* (mmHg)	Mean SAP (mmHg)	Diastolic SAP (mmHg)	Arterial pulse pressure (mmHg)	HR (beats/min)
0	137±18	118±15	98±12	39±8	173±28
0.002	137±15	118±13	98±11	39±6	185±31
0.008	133±18	117±16	93±13	39±5	198±33
0.01	134±16	113±14	92±12	42±5	182±24
0.02	138±19	113±16	89±13	45±6†	168±26
0.03	146±19	121±16	96±12	51±7†	176±27
0.05	154±20†	129±16	105±13	49±7†	196±28

*$p<0.05$ ANOVA; †$p<0.05$ Dunnett's test compared to baseline; †$p<0.01$ Dunnett's test compared to baseline, analysis of data recorded during the last minute (1 min) of infusion for each dose level.

Medetomidine is a selective alpha$_2$-adrenergic agonist which, following IV injection, produces an initial phase of hypertension followed by hypotension and bradycardia (Capuano, Lerche, & Valverde, 1999; Pypendop & Verstegen, 1998). This biphasic effect of medetomidine on SAP was also observed in our study. However, the degree of bradycardia (about 14% in this study) was 4.5 times lower than the one observed in dogs (decrease of 63%) for the same dose (Vanio & Palmu, 1989), and 3 times lower than for rhesus macaques with 0.05 mg/kg IV (decrease of 40%) (Capuano et al., 1999). This could be explained by the lower baseline value of HR in our monkeys. Also, we did not observe similar occurrence of sinus arrhythmias (complete sino-atrial block/sinus arrest with junctional escape beats, and infrequent ventricular premature contractions in 10 of 15 tested animals) (Capuano et al., 1999). Similarly to dogs, occasional second degree atrioventricular blocks were observed in the first 20 (Vanio & Palmu, 1989) to 30 min after injection in one monkey. In comparison, SAP was initially increased by 11 mmHg on average after IV administration of dexmedetomidine (0.002 mg/kg) to humans (Penttila, Helminen, Anttila, Hinkka, & Scheinin, 2004) compared with an increase of 29 mmHg in cynomolgus monkeys in the current study. Dexmedetomidine being the active molecule in the racemic mixture of medetomidine, we could extrapolate that the potential dose of dexmedetomidine in our study was 0.02 mg/kg. The 10 times higher dose administered to monkeys explains a greater,

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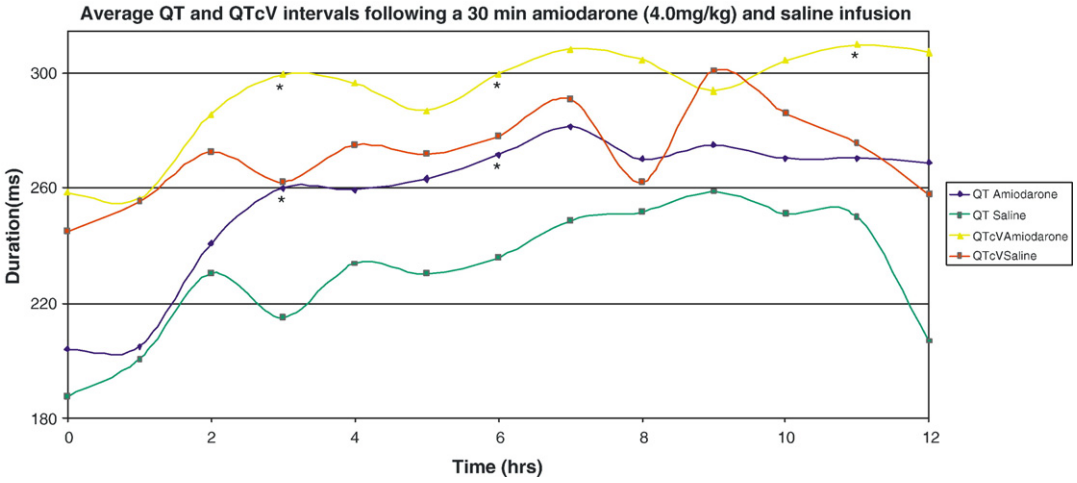

Fig. 3. Illustration of the effect of amiodarone infusion (0.4 mg/kg/min for 10 min) on QT and QTcV intervals in conscious telemetered cynomolgus monkeys ($n=4$). Amiodarone infusion was performed over a 30 min period ending at 1 h on the abscise axis of the above graph. ECG values obtained from the same monkeys at the same time of the day (6 pm to 6 am) prior to amiodarone administration were used as control for statistical analysis (*$p<0.05$ for paired comparison).

although not proportional, response magnitude. The effects on SAP were identical to those observed by Capuano et al. (1999) with a lower magnitude but a higher sensitivity related to the direct monitoring of SAP in our monkeys. Medetomidine was not selected as a positive control drug to evaluate QT interval prolongation but its ECG effect has been reported in other species (Kinjavdekar, Singh, Amarpal, Pawde, & Aithal, 1999). In our study, we observed significant QTcV and QTcF prolongation but no significant increase in QTcB.

Esmolol is a beta-adrenoceptor blocker reported to be cardioselective (beta$_1$-blocker), with no intrinsic sympathetic activity and low lipophilicity (Harrold, 1998). It produces dose-dependant bradycardia (Gorczynski, Murthy, & Hwang, 1984; Murthy, Hwang, Zagar, Vollmer, & Schmidt, 1983) associated to a decrease in velocity of atrioventricular conduction and a decrease in contractility. With larger doses, the relative selectivity to beta$_1$-adrenergic receptors is lost, and beta$_2$-receptors are also blocked, with potential bronchoconstriction, peripheral vasoconstriction, and decreased glycogenolysis (Opie, Sonnenblick, Frishman, & Thadani, 1995). Its extremely short half-life results from its hydrolysis by red blood cell esterase. A significant decrease in systolic, mean and diastolic SAPs combined with a decrease in pulse pressure and HR were identified with the telemetry system but the extent of the effect was limited to 10%–15% and only observed at the highest tested dose (2 mg/kg). This is indicative of a relatively good sensitivity of the telemetry system to detect slight but clinically relevant changes as observed with similar doses in humans (Kindler, Schumacher, Schneider, & Urwyler, 1996; Sintetos, Hulse, & Pritchett, 1987). Moreover, the more important effects observed on systolic and mean SAPs are suggestive of a prominent effect of esmolol on cardiac output (negative chronotropic and inotropic effects) (Berne & Levy, 1992).

Experimental hemodynamic studies in anesthetized dogs indicate that an IV injection of dopamine (0.001–0.009 mg/kg) induces a slight depressor response associated with a decrease in peripheral vascular resistance, a decrease in renal vascular resistance, an increase in renal blood flow, and an increase in cardiac output (McNay & Goldberg, 1966; Setler, Pendleton, & Finlay, 1975). The dopaminergic receptors in vascular smooth muscle (DA$_1$) subserves vasodilatory responses in the renal, visceral, coronary and cerebral beds (Chapman, Horn, Munday, & Robertson, 1980; Goldberg & Rajfer, 1985). High dose levels (0.009–0.018 mg/kg) produce pressor responses and a more pronounced increase in myocardial contractile force (beta$_1$-mediated chronotropic and inotropic positive effects) leading to increased systolic, mean and diastolic SAPs (Abdul-Rasool, Chamberlain, Swan, & Ward, 1987; Setler et al., 1975). Widening of pulse pressure is also reported in dogs (Robie & Goldberg, 1975). Considering that the highest dose tested (0.05 mg/kg/min) only induced a statistically significant increase in systolic SAP, it could be hypothesized that this effect was related to a beta$_1$ stimulation. Effects observed at lower doses most likely resulted from a beta$_2$ stimulation, leading to vasodilation, decreased total peripheral vascular resistance and diastolic SAP, as well as increased pulse pressure. In the dog, doses higher than 0.01 mg/kg/min present a risk of

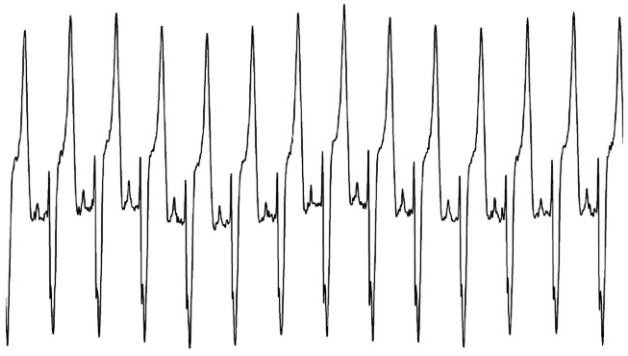

Fig. 4. Characteristic ECG changes induced by potassium chloride including tall peaked T-waves with widening of the QRS. This animal also presented a right bundle branch block.

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alpha-vasoconstrictor effect due to the loss of dose-dependent dopamine receptor selectivity. Based on our results, the vasculature of cynomolgus monkeys appears to be less sensitive than the dog to the pressor effects of dopamine, since the significant increase in systolic SAP (only observed at a dose of 0.05 mg/kg/min) is limited (approximately 10%). In humans, chronotropic and pressor effects of dopamine were significant in larger cohorts ($n=16$) of healthy volunteer at lower doses (0.003 mg/kg) (Marinac, Willsie, Dew, Pourakbar, & Herndon, 2001). These effects may also be significant in cynomolgus monkeys when using corresponding group sizes.

As a Class III anti-arrhythmic agent, amiodarone selectively prolongs the action potential and refractory period without effect on the resting membrane potential of the cell. This is clinically expressed as a prolongation of atrioventricular nodal conduction time and an increase in atrial and ventricular refractory periods (Novotny & Adams, 1986). Also, amiodarone induces QT interval prolongation after IV administration (Bertholet, Dubois, Materne, Demoulin, & Kulbertus, 1983) as well as a slight prolongation of the QRS complex (Cascio et al., 1988). The telemetered ECG monitoring reported all these effects. This wide range of ECG effects induced by amiodarone is a consequence of interactions with several cellular targets. Amiodarone blocks the rapid delayed rectifying potassium current (I_{Kr}), which prolongs the action potential and translates into QT interval prolongation. In addition, amiodarone blocks β-adrenoreceptors (Charlier, 1970) and several ion channels including calcium current (I_{Ca}) (Nishimura, Follmer, & Singer, 1989), sodium current (I_{Na}) (Follmer, Aomine, Yeh, & Singer, 1987) and several potassium current such as I_{Ks}, I_{to}, I_{K1}, I_{KACh} and I_{KNa} (Yoshida et al., 2002). Despite very interesting pharmacodynamic properties and its popularity as a cardiovascular drug (Siddoway, 2003), amiodarone presents a highly variable pharmacokinetic profile with an elimination half-life averaging 58 days in humans. These characteristics may be considered prohibitive for some validation studies where animals need to be reused in subsequent studies after an appropriate wash-out period. Given these considerations, specific I_{Kr} blockers with shorter half-life such as sotalol (Poirier et al., 1990) or dofetilide (Ollerstam, Visser, Persson, Eklund, Nilsson, Forsberg, 2006) may be preferred as positive controls for QT prolongation in validation studies.

The infusion of potassium chloride was used to verify the ability of the system to detect ECG morphology changes associated with hyperkalemia. Hyperkalemia lowers the resting membrane potential of excitable cardiac cells and decreases the duration of the myocardial action potential and upstroke velocity. This decreased rate of ventricular depolarization, plus the beginning of repolarization in some areas of the myocardium while other areas are still undergoing depolarization, produces characteristic ECG changes: peaked T-waves, widening of the QRS complex that merges with the T-wave into a sine wave appearing at severely elevated levels, and loss of P-waves (Mattu, Brady, & Robinson, 2000). The earliest manifestations of hyperkalemia are narrowing and peaking of the T-wave. Though not diagnostic of hyperkalemia (Somers et al., 2002), T-waves are almost invariably peaked and narrowed when serum potassium levels range between 7 and 9 mEq/L. The telemetered ECG system detected this early change in all monkeys. When serum potassium levels exceed 7 mEq/L, atrial conduction disturbances appear as a decrease in P-wave amplitude and an increase in PR interval. Supraventricular tachycardia, atrial fibrillation, premature ventricular complexes, ventricular fibrillation, or sinus arrest may occur. We did observe in the first cynomolgus monkey (longer infused with the potassium solution) losses of P-waves and atrial conduction disturbances. This is also a beneficial element with regards to the system sensitivity to detect ECG arrhythmias.

Interpretation of regulatory guidelines in the preclinical industry was the basis for selection of our validation study design. In response to regulatory guidelines, preclinical laboratories working under GLP regulations will elaborate validation protocols, which will differ to a certain extent from one laboratory to another. Comparison of study design and results among laboratories is often not possible due to the legal environment in which contractual research organizations and pharmaceutical companies evolve. Based on our results, several elements were considered important for validation of this test system. Validation of both numerical values and ECG tracing in normal and positive control drug treated animals was central to confirm accuracy of this study endpoint. Results from the validation allowed definition of precise experimental conditions in which this test system could operate. As an example, computerized ECG analysis provided accurate interval measurement in saline-treated cynomolgus monkeys. In presence of severely increased HR following drug administration, analysis with the software revealed that, with standard settings, the software was unable to identify individual complexes. This observation prompted the need to have all complexes used for computerized ECG analysis reviewed by trained personnel. In conclusion, this validation study was pivotal to the preparation of standard operating procedures to ensure that the test system would be used within conditions that ensure accurate and reliable safety pharmacology evaluations. The design of the current validation is considered adequate as an initial validation. More drug responses may be useful to better characterize the sensitivity of the model and more animals would be needed to further assess drug reproducibility.

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