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Original article

A cardiovascular monitoring system in conscious cynomolgus monkeys for regulatory safety pharmacology Part 1: Non-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:** The validation provided by the supplier evaluated installation (IQ) and operation (OQ) qualifications. This protocol was completed with tests evaluating electronic data management and accuracy and precision of transmitter ($n=4$) measurements for temperature and pressure criteria with a series of tested values. As part of performance qualification, physical activity (for 24 h) as well as cardiovascular, ECG (20 complexes for each animal) and systemic arterial blood pressure (SAP, 10 different measures), data were recorded simultaneously from the same animals ($n=4$) using certified equipment and the telemetry system. Reliability was evaluated over 60 days. **Results:** The IQ and OQ were completed successfully. The electronic data management was performed successfully. The *ex-vivo* evaluation for temperature and pressure showed high correlation ($R^2>0.99$) but with a slight pressure shift, as expected, with this transmitter model. For physical activity, the correlation coefficients were good to excellent with high activity counts but the comparison demonstrated a limited sensitivity of the telemetry system with animal presenting low activity levels. ECG interval measurement using the telemetry software was considered at least equivalent to manual measurement, but with some limitations in the reading of the ECG. The comparison between both methods of SAP measurement showed adequate precision ($R^2=0.969$) but no accuracy. **Discussion:** Reference monitoring methods are important to ensure proper test system validation. Monitoring with a reference methodology and the telemetry system is important in order to evaluate precision and accuracy of the test system. Computerized analysis may lack the capability to analyze ECG complexes with abnormal morphologies. This reinforces the need to have ECG evaluation prior to telemetry implantation along with visual evaluation of ECG tracing at standard speed (*e.g.* 50 mm/s) at all time points.

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

1. Introduction

The importance of telemetric monitoring in biomedical research involving laboratory animals has grown significantly over the past years. This technology is an important tool for collection of a considerable number of physiological parameters including electrocardiograms, electroencephalograms, electromyograms, arterial blood pressures, ventricular blood pressures, locomotor activity, core body temperature and pleural pressures (Brockway, Mills, & Kramer, 1998). For researchers, especially those in the fields of pharmacology and toxicology, telemetry provides a valuable tool to define the physiological and pathophysiological consequences derived from advanced molecular, cellular, and tissue biology and to predict new compound effectiveness and safety in humans. Particularly, systemic arterial

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pressure (SAP), heart rate (HR) and electrocardiography (ECG) should be evaluated as part of preclinical safety pharmacology (US FDA — ICH S7A, 2001, 2004).

If continuous measurement of cardiovascular parameters in experimental animals is essential for cardiovascular research, species selection for safety pharmacology and drug toxicity testing is important in order to develop new clinically useful pharmaceuticals. Historically, canine models were most frequently used for cardiovascular safety pharmacology studies when large laboratory animals were required. Some considerations for selection of relevant preclinical models have justified sensitivity evaluation and validation of telemetered non-human primate models (Chaves et al., 2006). New methods for correcting QT interval for HR have been recently evaluated in cynomolgus monkey (Holzgrefe et al., in press). Data obtained in conscious cynomolgus monkeys have also been published recently (Ando et al., 2005; Gauvin, Tilley, Smith, & Baird, 2006). The pharmacokinetics of xenobiotics in humans is closer to non-human primates than to dogs for a number of drugs (Ward & Smith, 2004). *In-vivo* metabolism also supports the use of non-human primate models for various drugs as metabolites may be responsible for adverse cardiovascular effects (Fermini & Fossa, 2003). Metabolism in non-human primates is closer to human than dog for some drugs (Zuber, Anzenbacherova, & Anzenbacher, 2002). Reducing the use of non-human primates in research is an important overall objective from both an ethical and resource perspective. However, the high degree of relevance of monkeys for some drugs makes it a judicious model for cardiovascular safety pharmacology evaluation, when justified.

In light of studies described in the literature (Akita, Kuwahara, Nishibata, Mikami, & Tsubone, 2004; Kramer et al., 2000; Harkin, O'Donnell, & Kelly, 2002; Schierok, Market, Pairet, & Guth, 2000; Schlatter & Zbinden, 1982), it was concluded that the use of radiotelemetry to measure SAP, ECG, HR, body temperature and locomotor activity in rodents has been sufficiently validated (Kramer et al., 2001; Kramer & Remie, 2005; Shiotani, Harada, Abe, Hamada, & Horii, 2007). Data on circadian rhythms of SAP (Gauvin et al., 2006; Gerber, Schnell, & Anzenberger, 2000; Schnell & Wood, 1993), HR (Gauvin et al., 2006; Gerber et al., 2000; Schnell & Wood, 1993), and body temperature (Cilia, Piper, Upton, & Hagan, 1998; Gauvin et al., 2006; Palkova, Sigmund, & Erkert, 1999) in marmoset and cynomolgus monkeys have been reported in the literature. For the latter, most of the data have only been reported in abstracts of annual meetings (Kamenosono, Hamada, Fukuzaki, Nagayama, & Kito, 1999; Kito, Kamenosono, Akune, Fukuzaki, & Nagata, 1999), or using a simple Holter monitor (Macallum & Houston, 1993). Even if non-human primates are used routinely for regulatory cardiovascular safety pharmacology, validation study results are rarely reported in the scientific literature and data available is often limited (Ando et al., 2005; Omata, Kasai, Hashimoto, Hombo, & Yamamoto, 2005). Validation of electronic technologies to generate electronic records and electronic signatures has been the subject of significant discussions among interested parties following issuance of the final Code of Federal Regulation 21 Part 11 (US FDA — CFR 21 Part 11, 1997). As a result of these concerns, FDA issued a guidance document providing insights on FDA interpretation of Part 11 requirements (US FDA — CFR 21 Part 11, 2003). This guidance emphasizes the importance of using a documented risk assessment to determine the extent of system validation.

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.

2.2. DSI validation protocol

First, the supplier (DSI) performed a series of tests (GLP Large Animal Validation Protocol) developed for the validation of the system based on 21 CFR Part 11 and Part 58. The validation protocol performed on-site by the supplier included installation and operation qualifications. Tests performed by the supplier evaluated all recording and analysis functions of the software in the absence of animals. Security checks and audit trails were also tested. Lastly, a transmitter simulator (TSS-1™, DSI) producing a signal with known characteristics was used to validate accuracy of radiowave signal capture.

2.3. Electronic data management

The ability to generate accurate and complete copies of electronic records is critical to allow proper interpretation of experimental results and is a requirement of 21 CFR Part 11.

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Samples of the electronic raw data (6080 values) were printed directly from the telemetry system software. The same electronic data was imported using Microsoft Excel software (Microsoft Canada Co., Mississauga, ON, Canada). Then, the Excel documents were converted into a PDF document (Adobe Acrobat Professional 5.0, San Jose, CA, USA) and printed. The printed raw data was compared to the printed Adobe Acrobat data for quality control of data transfer. A sample of the electronic study data was also transferred to archives (DVD) and integrity was evaluated after 1 week and 6 months.

2.4. Ex-vivo evaluation

Ex-vivo tests evaluated transmitter precision and accuracy in controlled conditions without animal. These tests are equivalent to standard curves used with most measurement systems.

2.4.1. Temperature

Four transmitters (TA10TAD70™, DSI) were placed consecutively in a beaker containing water at temperatures within the physiologically possible range (34 °C to 40 °C). The temperature of the water was recorded simultaneously every minute for at least 20 min using a calibrated digital thermometer (016-605™, AMG Medical Inc., Montreal, QC, Canada) and the telemetry system.

2.4.2. Pressure

Four transmitters (TL11M2-D70-PCT™, DSI) were placed consecutively in a pressure chamber (DSI, St-Paul, MN, USA). The pressure in the chamber was monitored simultaneously using a calibrated manometer (DPM-1b, Biotek®, Winooski, VT, USA) and the telemetry system. Pressure increments of 25 mm Hg from 0 to 250 mm Hg were applied. For each pressure increment, the values obtained from the manometer and telemetry system were recorded every minute for 10 min.

2.5. In-vivo evaluation

During the study, the care and use of animals were conducted in accordance with the principles outlined in the current Guide to the Care and Use of Experimental Animals as published by the Canadian Council on Animal Care and the Guide for the Care and Use of Laboratory Animals, a National Research Council publication. LAB Research Inc.'s facility is AAALAC accredited.

Four cynomolgus monkeys (*Macaca fascicularis*), 2 males and 2 females, were used for ECG, SAP and locomotor activity evaluations. 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.

2.5.1. Anesthesia

Animals were anesthetized with an intramuscular (IM) injection of acepromazine (Atravet®, 10 mg/ml, 0.14 mg/kg, Ayerst, Guelph, ON, Canada) and ketamine (Ketaset™, 100 mg/ml, 13.6 mg/kg, Ayerst, Guelph, ON, Canada). Lidocaine spray (Lidodan™, 10% w/w, Odan Laboratories Ltd., Pointe-Claire, QC, Canada) was administered onto the arytenoids prior to endotracheal intubation using laryngoscopy. A sterile ophthalmic ointment (Duratears®, Alcon Canada Inc., Mississauga, ON, Canada) was applied to both eyes to prevent drying of the cornea. Animals were then placed on a heating pad and inhaled a mixture of oxygen (O_2) and isoflurane (AErrane™, Baxter Corporation, Mississauga, ON, Canada) with the O_2 flow meter and the vaporizer set approximately at 1.0 l/min, and 2.5%, respectively. Respiratory rate was maintained between 10 and 12 breaths/min with an inspiratory airway pressure between 18 and 20 cm H_2O using a mechanical ventilator (2002, Hallowell EMC, Pittsfield, Massachusetts, USA). Monitoring during anesthesia included HR and pulsatile hemoglobin saturation in O_2 (VetOx 4404™ pulse oximeter, Heska™, Fribourg, Switzerland). Prophylactic antibiotic therapy (Cefazolin injectable, 25 mg/kg, Novopharm®, Toronto, ON, Canada) was administered by IM injection at least 1 h prior to surgery, at the end of surgery and every 8 h for 24 h post-surgery. Analgesic (buprenorphine, Temgesic™, 0.05 mg/kg, Schering-Plough, Welwyn Garden City, Hertfordshire, United Kingdom) was administered by IM injection upon completion of the surgery and every 8 h for 24 h post-surgery. Fluid therapy was given intravenously (IV) throughout anesthesia using sterile Lactated Ringer's solution at a rate of 10 ml/kg/h. The surgical site was shaved and aseptically prepared using chlorhexidine gluconate 4% and isopropyl alcohol.

2.5.2. Transmitter implantation

An incision parallel to the *linea alba* was made in the abdominal wall to allow D70-PCT™ (DSI) transmitter placement. The latter was inserted between the abdominal internal oblique and the abdominal transverse muscles through a longitudinal incision in the middle of *rectus abdominis* muscle. A surgical approach to the right inguinal region gave access to the femoral artery. The monitoring catheter was tunneled subcutaneously to the inguinal incision using a trocar. An arteriotomy was performed and the monitoring catheter was inserted into the femoral artery. After securing the monitoring line with non-absorbable sutures (Polybutester 4-0, Novafil™, Tyco Healthcare Group LP, Norwalk, CT, USA) the surgical sites were flushed with warm sterile saline. Then, skin incisions were made on the left lateral aspect of the thorax just cranial to the last rib and on the right side of the thorax in the area of the thoracic inlet, to allow ECG lead placement (DII). The skin incisions were closed with interrupted intradermal buried sutures using absorbable suture material (Polyglactin 3-0, Vicryl™, Ethicon Inc., Somerville, New Jersey, USA). This suture pattern ensured that no monkey could easily remove any suture.

An incision was made in the left inguinal region for femoral vein infusion catheter placement. A second incision was made in the interscapular region for catheter exteriorization. The catheter

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was secured using non-absorbable suture material (Polypropylene 3-0, Prolene™, Ethicon Inc., Somerville, New Jersey, USA). The surgical sites were irrigated with sterile warm saline. A loop of catheter was secured into a s.c. skin pocket made in the inguinal region. The incision was closed with interrupted buried sutures using absorbable material (Polyglactin 3-0, Vicryl™). Following surgery, each animal was equipped with a jacket and tether system. Rectal body temperature was monitored in the post-operative period until animals reached at least 37.0 °C, where they were returned to their cage.

2.5.3. Procedures

2.5.3.1. Physical activity monitoring. Locomotor activity was monitored simultaneously using the telemetry system and an external physical activity monitoring device (Actical™, Mini Mitter®, Bend, OR, USA) was attached to the jacket of each cynomolgus monkey for a period of at least 24 h. Upon completion of the monitoring period, the data from the external device was uploaded and compared to the DSI telemetry data. To allow comparison, the sum of activity intensity for each animal, for each hour, obtained from DSI and Actical™ systems were used for statistical analysis.

2.5.3.2. Cardiovascular monitoring

2.5.3.2.1. Electrocardiography. Electrocardiograms from 4 conscious restrained cynomolgus monkeys were recorded simultaneously with the telemetry system and with a medical electrocardiograph (MAC 1200™, GE Medical System IT Inc., Milwaukee, WI, USA) for a period of at least 1 min on 2 different occasions. The following parameters were evaluated: PQ, PR, QRS, QT, RT, RR, RTp (R to T peak) and Tpe (peak to end) intervals, ST segment, QTd (QT dispersion), and HR (in beats per min, bpm). Parameters from 10 complexes recorded with the MAC 1200™ electrocardiograph, distributed in each 1-min period, were manually calculated by a veterinarian. The electrocardiogram ruler used for manual interval measurement had a precision of 0.02 s at 50 mm/s. Parameter averages (2 occasions × 10 complexes × 4 monkeys = 80 values) manually calculated were compared with intervals measured by the software for the same period. All ECG marks placed by the software for each 1-min period were reviewed prior to interval computation to ensure adequate complex processing.

2.5.3.2.2. Systemic arterial blood pressures. Blood pressures (systolic, mean and diastolic SAP) were measured using a non-invasive electronic oscillometric sphygmomanometer (Minipack 911™, Pacetech Inc., Clearwater, FL, USA) on 10 different occasions in 2 cynomolgus monkeys. The oscillometric sphygmomanometer pediatric cuff was placed on a thoracic limb (middle third of the humerus) while direct arterial pressure was measured by the telemetry system through the catheter in the femoral artery.

2.6. Statistical methods

Simple linear regression (SPSS, Chicago, IL, USA) was performed on both *ex-vivo* (temperature and pressure) and *in-vivo* (physical activity, SAP) data. Pearson's correlation coefficient (R^2), slope and origin ordinate were used to evaluate accuracy and detect measurement shifts between measuring methods. Statistical tests were performed at the 0.05 threshold of significance. Mean ± SD data are presented.

2.7. Acceptance criteria

Complete integrity of electronic data must be preserved to consider electronic data management acceptable. Given the experimental conditions for *ex-vivo* temperature and pressure evaluations (beaker containing water and pressure chamber) and the precision of reference measurement methods (0.1°C and 1 mm Hg), maximal differences of 1 °C and 2 mm Hg were considered acceptable for temperature and pressure evaluations, respectively. Electrocardiographic analysis performed using the Physiostat™ ECG Analysis version 4.01 software was considered accurate if the maximal difference between computed and manual ECG analysis was less than 0.03 s, which is 1.5 times the precision of the electrocardiographic ruler (0.02 s) used for manual interval measurements. For SAP, correlation was a more important criterion than accuracy between both used methods of measurement. Reliability and consistent intended performance as required by 21 CFR Part 11 were evaluated throughout the study conducted over a 60-day period.

3. Results

3.1. DSI validation protocol

Tests performed by the supplier included installation and operation qualifications. Verification that all software recording and analysis functions were correctly operating, as per specifications in the absence of living animals, was done. Security checks and audit trails were also successfully tested. Lastly, the average of 10 cycles of the signal simulator was confirmed to be within the range specified in the acceptance criteria for temperature, HR (pressure channel), systolic, mean and diastolic pressure, HR (ECG), pulse pressure and activity.

3.2. Electronic data management

All numerical values were accurately processed into Adobe Acrobat tables from the electronic raw data. The integrity of the archived electronic raw data was preserved and copies could be generated. The electronic raw data could not be altered by users or the system administrator. The electronic raw data could be deleted from the computer by the administrator but could not be deleted from the archived media (DVD). When the internal clock of the computer was adjusted by the administrator to a previous time where telemetry data was recorded, no overwriting was possible. If the original electronic raw data files had been deleted, new electronic raw data could be retrospectively generated at the exact same time as the study was conducted but this fraud could be detected through the audit trail, which could not be altered by the administrator.

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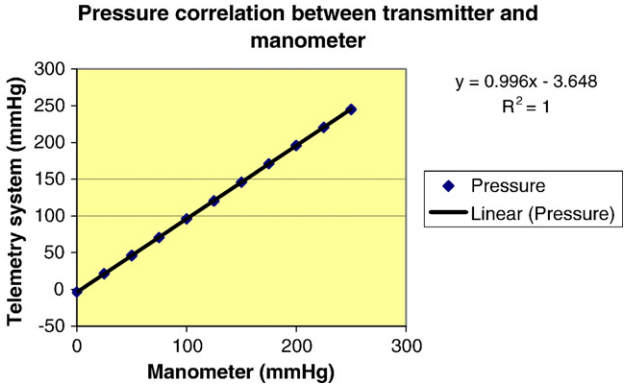

Fig. 1. Pressure correlation curve comparing the telemetry system and the reference manometer (*ex-vivo* evaluation).

3.3. Ex-vivo evaluation

3.3.1. Temperature

The correlation coefficients of simple linear regressions were between 0.999 and 1 for the 4 transmitters evaluated. Differences between temperatures from the digital thermometer and the telemetry system ranged from −0.3 to 0.3 °C. The slopes obtained from the 4 transmitters were between 1.002 and 1.008. Therefore, increments measured by the calibrated digital thermometer were considered accurately quantified by the telemetry system. The values of origin ordinates ranged between −0.200 and −0.456 °C.

3.3.2. Pressure

Correlation coefficients for pressures measured with a calibrated manometer and the telemetry system were maximal ($R^2 = 1.00$) for all transmitters evaluated. The origin ordinate of linear regression functions ranged between −3.648 and −4.342 mm Hg, indicating a negative pressure shift (Fig. 1). The linear regression slopes ranged between 0.996 and 1.005.

3.4. In-vivo evaluation

3.4.1. Physical activity monitoring

The correlation coefficients of linear regression ranged from 0.51 to 0.96. Better correlation ($R^2 > 0.9$) was noted for animals with high activity counts. The absence of activity count was frequent with the telemetry system, particularly when the monkeys were less active.

3.4.2. Cardiovascular monitoring

3.4.2.1. Electrocardiography. The ECGs of 3 of the 4 animals could be analyzed with the computerized telemetered system. Standard and telemetry ECG confirmed that 1 animal had reduced R-wave amplitude with a deep S-wave in derivation II (Fig. 2). The Physiostat™ ECG Analysis software was unable to measure intervals and amplitudes of complexes with reduced R-wave amplitude. A fifth cynomolgus monkey with normal QRS complexes (normal R-wave amplitude) was surgically prepared with a telemetry transmitter and was used to complete the validation protocol.

Manual (ECG ruler) and computerized interval measurements for 1-min periods revealed a difference of −14.1 to 25.8 ms. Comparison for QTd was not considered relevant given that the precision of the ECG ruler was less than the expected QTd (<0.02 s). Mean HR were 181±14 and 178±15 bpm for manual and telemetry measurements, respectively.

3.4.2.2. Systemic arterial blood pressures. A correlation coefficient of 0.969 was present between systolic SAP values evaluated with the automated sphygmomanometer and the telemetry system. All values obtained with the sphygmomanometer were lower than those obtained with the telemetry system. The average indirect systolic SAP was 139 mm Hg compared to 150 mm Hg for the telemetry system.

4. Discussion

This study demonstrated that the installation and operation qualifications of the DSI telemetered cardiovascular monitoring system were successful. Particularly, electronic data transfer, security checks, audit trails, signal tests, electronic signature, archive and measurement precision and accuracy were verified with *ex-vivo* tests that were designed to be representative of standard operating procedures with normal and abnormal (intentional or not) use of the system. The *ex-vivo*

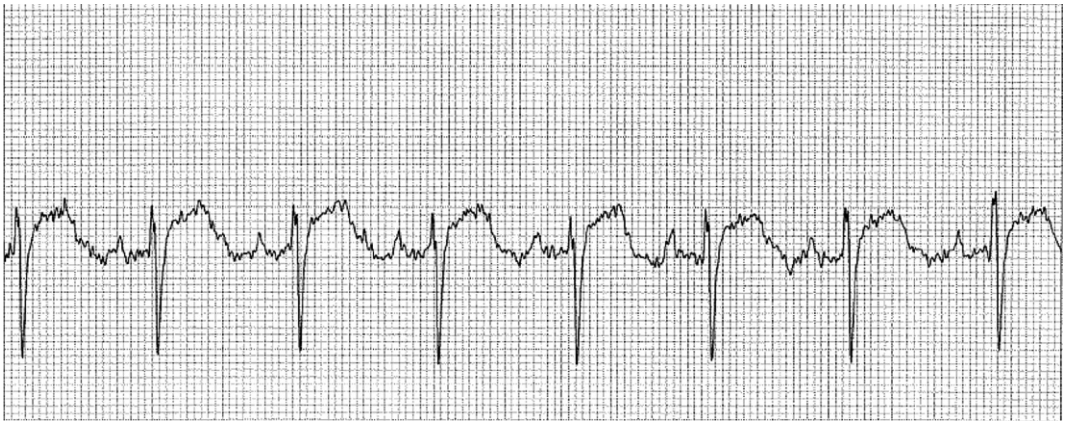

Fig. 2. Electrocardiographic tracing of a cynomolgus monkey with reduced R-wave and deep S-wave in derivation II.

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evaluation tested, in reference to calibrated monitors, the temperature and the pressure measurement precision and accuracy. Precision is how repeatable the measurements are. Accuracy is how close a value is to the true value. An inaccurate, but precise monitor can be re-calibrated, but an imprecise monitor cannot be improved (Szocik, Barker, & Tremper, 2005). For both variables, the correlation coefficients of simple linear regressions were excellent. The differences observed for absolute temperature values (from −0.3 to 0.3 °C) and for the origin ordinate (between −0.200 and −0.456 °C) were not considered clinically significant. Both reference and tested thermometers were thermistor probes and their comparison showed them to be precise. The pressure increments, measured by the telemetry system and the reference manometer, were considered equivalent but the presence of a possible pressure shift (Fig. 1) should be evaluated for telemetry transmitters prior to implantation and once the transmitter is removed at necropsy.

During the *in-vivo* evaluation, the procedures completed the comparative validation of accuracy and precision for physical activity monitoring, ECG and SAP measurements. Briefly, the DSI telemetry device was found to be less efficient than the Actical™ external monitor for locomotor activity monitoring. In contrast, evaluation of ECG and SAP with the comparative methods (manual ECG ruler, and non-invasive oscillometric sphygmomanometry of SAP) showed evidences of limitations compared to the telemetry system. From a surgical perspective, the use of buried suture for skin incision eliminated post-operative complications due to removal of sutures by non-human primates. The suture pattern used in the current study was used in 118 telemetry implantation surgeries in non-human primates over the past 2 years in our laboratory with no case of wound dehiscence or infection (Authier et al., 2006, unpublished observation).

For the locomotor activity counting, the correlation coefficients were good to excellent with high activity counts. When the monkeys were less active, inducing less signals, activity count absence was more frequent with the DSI telemetry system, which lead to poor correlation coefficient value. With lower activity, the locomotor activity analysis is more limited. This highlights a limited sensitivity for the DSI device, when compared to the Actical™ monitor. Based on these results, interpretation of locomotor activity data from this telemetry system should be done with care and activity should only be used as a complement to interpretation of the data of interest (cardiovascular or core body temperature).

Only monkeys presenting normal ECG complexes including the presence of normal amplitude R-waves should be used for ECG evaluation when using the Physiostat™ ECG Analysis software. An ECG evaluation is required prior to surgical preparation of the animals and all time points evaluated following drug administration should include a sample ECG at standard speed (*e.g.* 50 mm/s). Differences observed with intervals measurement are considered mainly attributable to the measurement precision of the ECG ruler (20 ms). Differences could also result from the limited number of complexes evaluated manually (10 complexes) in each 1-min period compared to evaluation of all complexes with the telemetry system. Given these results, electrocardiographic interval measurement using the Physiostat™ ECG Analysis software is considered at least equivalent to manual measurement when all complexes included in the analysis are reviewed for accuracy of computerized interval mark placement.

Common sources of error during non-invasive oscillometric sphygmomanometry include selection of an inappropriate cuff size. The width of the SAP cuff should be 20% greater than arm diameter or 40% of the limb circumference, and it should be applied snugly after any residual air has been squeezed out. Although a too large cuff will generally work well and produce little underestimating error, the use of cuffs that are too narrow will result in an overestimation of SAP. The American Association for the Advancement of Medical Instrumentation standards requires that a monitor records SAP within a 5 ± 8 mm Hg (mean ± SD) prediction error with respect to the reference method (Weiss & Pasch, 1997). Even though automated non-invasive SAP measurement techniques are considered non-invasive and relatively safe, complications have been reported. These morbid events include pain, petechiae and ecchymoses, limb edema, venous stasis and thrombophlebitis, peripheral neuropathy, and even compartment syndrome (Sutin, Longaker, Wahlander, Kasabian, & Capan, 1996; Weiss & Pasch, 1997). They occur more often after prolonged periods of excessively frequent cuff inflations/deflations cycling, are visible in critically ill patients, particularly in humans, and are due to trauma or impaired distal limb perfusion. In animals, these complications are rare, but cuff displacement may occur in conscious monkeys. Our results reinforce the need to compare values recorded with the same measuring method to obtain valid conclusions. The widespread application of invasive SAP monitoring in anesthesia and intensive care is also related to the good safety record of the technique. Large clinical investigations confirm the low incidence of long-term complications after distal artery cannulation, in particular, the small risk of distal ischemia, which is estimated at less than 0.1% (Mandel & Dauchot, 1977). Other complications of direct SAP monitoring include hemorrhage, arterial embolization, infection, peripheral neuropathy and most commonly, misinterpretation of data or misuse of equipment (Cockings, Webb, Klepper, Currie, & Morgan, 1993; Mandel & Dauchot, 1977).

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. In addition to the design and results that are presented in the current study, several elements were considered important and are presented below. The use of proper reference measurement methods (thermometer, manometer, external physical activity counter, oscillometric sphygmomanometer and certified external ECG) was important to evaluate the accuracy and precision of the system.

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