Journal of Pharmacological and Toxicological Methods 81 (2016) 120–127

Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

journal homepage: www.elsevier.com/locate/jpharmtox

Research article

Rat cardiovascular telemetry: Marginal distribution applied to positive control drugs

M.V. Accardi [a], E. Troncy [b], S. Abtout [a], A. Ascah [a], S. Maghezzi [a], S. Authier [a,b,*]

[a] CiToxLAB North America, 445 Armand Frappier, Laval, QC H7V 4B3, Canada
[b] Faculty of Veterinary Medicine, University of Montreal, P.O. box 5000, St-Hyacinthe, QC J2S 7C6, Canada

ARTICLE INFO

Article history:
Received 17 February 2016
Received in revised form 25 March 2016
Accepted 27 March 2016
Available online 31 March 2016

Keywords:
Telemetry
Rat
Contractility
Left ventricular
Cloud analysis
ECG
PR
QT

ABSTRACT

Cardiovascular effects are considered frequent during drug safety testing. This investigation aimed to characterize the pharmacological response of the conscious telemetered rat *in vivo* model to known cardiovascular active agents. These effects were analyzed using statistical analysis and cloud representation with marginal distribution curves for the contractility index and heart rate as to assess the effect relationship between cardiac variables. Arterial blood pressure, left ventricular pressure, electrocardiogram and body temperature were monitored. The application of data cloud with marginal distribution curves to heart rate and contractility index provided an interesting tactic during the interpretation of drug-induced changes particularly during selective time resolution (*i.e.* marginal distribution curves restricted to T_{max}). Taken together, the present data suggests that marginal distribution curves can be a valuable interpretation strategy when using the rat cardiovascular telemetry model to detect drug-induced cardiovascular effects. Marginal distribution curves could also be considered during the interpretation of other inter-dependent parameters in safety pharmacology studies.

1. Introduction

Cardiovascular monitoring methods using telemetry have become an important component in preclinical safety assessments over the last decade, providing pharmacologists a simple but valuable tool to evaluate drug safety. Telemetry is widely employed to monitor a variety of cardiovascular parameters such as blood pressure, heart rate (HR), electrocardiogram (ECG), and body temperature (BT), amongst others, in awake, and freely moving laboratory animals (Kramer and Kinter, 2003). These systems are typically employed in regulatory safety pharmacology studies as detailed in the S7A guideline from the International Conference of Harmonization (ICH) (FDA, 2001). The use of telemetry systems in safety assessments has since seen comprehensive implementation and validation in a variety of preclinical animal models (*e.g.*, rodents, minipigs, dogs and nonhuman primates) (Authier et al., 2007a, 2007b; Authier et al., 2011; Deveney et al., 1998; Gauvin et al., 2006; Gelzer and Ball, 1997; Kramer and Kinter, 2003; Markert et al., 2009; Segreti et al., 2016; Shiotani et al., 2007). While a broad range of species is available, the rat remains the most frequently used model for regulatory toxicology assessments (Gad, 2012) and a well-established preclinical cardiovascular safety testing model (Guth, 2007; Jacob, 2010). Apart from the obvious difference in size, the human and rat heart share similar morphological and physiological features including similar systolic, mean and diastolic pressures (Wessels and Sedmera, 2003). Moreover, the mRNA and expression profile of major cardiac ion channel proteins in both the atria and ventricle of rats and humans are also similar with the noted exception of the I_{Kr} or human *ether-à-go-go*-related gene potassium channel (hERG)-like current which is absent in rats (Wymore et al., 1997). The use of telemetry cardiovascular monitoring in rats has been employed and successfully validated (Brockway, Mills, and Kramer, 1998; Deveney et al., 1998; Kramer et al., 2001; Kramer and Kinter, 2003; Kramer and Remie, 2005) demonstrating expected cardiovascular and ECG modifications in responses to various cardioactive agents. However, the available scientific literature with rat telemetry presents limited consideration for agents inducing inotropic effects. Given this, we aimed to assess the pharmacodynamics response of telemetered rats to known pharmacological agents and document the corresponding electrocardiographic, hemodynamic, chronotropic and inotropic effects. In doing so, we provide a better understanding of this pre-clinical model as well as offer a qualitative approach to beat-to-beat hemodynamic data presentation through the use of marginal distribution curves.

* Corresponding author at: CiToxLAB North America, 445 Armand Frappier, Laval, QC H7V 4B3, Canada.
E-mail address: authiers@ca.citoxlab.com (S. Authier).

http://dx.doi.org/10.1016/j.vascn.2016.03.005

2. Materials and methods

2.1. Statement on use and care of animals

During this investigation, 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. CiToxLAB's facility is AAALAC accredited and the procedures were reviewed and approved by the Institutional Animal Care and Use Committee prior to conduct. All procedures were conducted as per Standard Operating Procedures (SOPs) in place.

2.2. Animals and environment

All animals were housed under standard laboratory conditions with controlled temperature (21 ± 3 °C), humidity (30%–70%), 12 h light/dark cycle and 10–15 air changes per hour. Temperature and relative humidity were monitored continuously. The animals were provided a standard certified commercial chow (Harlan Teklad Certified Global Rodent Diet #2018C) and municipal tap water (which has been exposed to ultraviolet light and purified by reverse osmosis) *via* water bottles *ad libitum*. Male Sprague–Dawley rats (Charles River Laboratories, St-Constant, QC), aged approximately 22 weeks old and weighing approximately 500 g at the beginning of the study were used.

2.3. Surgical instrumentation

2.3.1. General anesthesia and surgical preparation

Penicillin G procaine (Procillin, 300,000 IU/mL, Vetoquinol, Lavaltrie, Quebec, Canada) and Buprenorphine (Temgesic™, 0.05 mg/kg, Schering-Plough, Welwyn Garden City, Hertfordshire, UK), were administered by subcutaneous injection prophylactically. General anesthesia was induced with 2–4% oxygen-isoflurane (AErrane™, Baxter Corporation, Mississauga, ON, Canada) mixture and tracheal intubation was done with mechanical ventilation at a respiratory rate of 60–85/min and tidal volume of 2–2.5 ml. Anesthesia was maintained for the duration of the surgery and rats were closely monitored for the depth of anesthesia. Rats were placed on a heated surgical field in dorsal recumbency. The surgical sites were shaved and the skin was aseptically prepared and draped with sterile gauze.

2.3.2. Surgical procedure

All animals underwent surgery for telemetry transmitter implantation (Data Science International (DSI), Model DSI HD-S21) to monitor blood pressures, ECG, BT and locomotor activity. Surgeries were performed by a ventral midline incision on the *linea alba* using aseptic techniques. The telemetry transmitter was implanted in the left upper quadrant of abdominal cavity, parallel to the long axis of the body. The transmitter body was fixed to the abdominal wall using suture ribs (4–0 polypropylene). Local analgesics, bupivacaine (0.25%, 0.05 ml; Hospira, Montreal, Quebec, Canada) and lidocaine (20 mg/ml, 0.05 ml; Lurocaine, Vetoquinol, Lavaltrie, Quebec, Canada), were mixed and injected subcutaneously at the abdominal incision. The arterial blood pressure catheter was inserted into one of the iliac arteries and the electrocardiographic leads were implanted subcutaneously across the sternum in a Lead II configuration. For left ventricle pressure catheter placement, a 3–0 nylon suture was inserted through the xiphoid and retracted anteriorly to lift the thoracic cavity to allow maximum exposure of the diaphragm. A midline vertical incision was made in the diaphragm and a 5–0 propylene suture was passed through each side to retract the diaphragm, exposing the heart. The apex of the heart was located and the left ventricle was subsequently punctured with a 23 G needle and the tip of the telemetry pressure catheter was inserted into the ventricle up to the suture rib (approx. 8 mm). The catheter was fixed in position by tightening the purse string suture and secured. Once the catheters and lead placements were finalized, the diaphragm was sutured ensuring that the catheter exited the thoracic cavity through the diaphragm at the dorsal end of the incision. Air was aspirated from the thorax *via* a 25 G butterfly attached to 5 ml syringe inserted in the intercostal space. Negative pressure was re-established prior to removal of the syringe.

2.3.3. Post-surgical recovery

The abdominal site was flushed with warm saline and the incisions were closed with absorbable suture material (Novafil 4-0; Covidien, Saint-Laurent, QC) using simple continuous sutures. The skin was closed with discontinuous buried sutures using absorbable suture material (Vicryl Polyglactin 4-0; Ethicon, Johnson & Johnson, Somerville, NJ, USA). Instrumented rats were single housed in their home cages and telemetry recordings commenced immediately. Penicillin G procaine and buprenorphine were subcutaneously injected BID for two days following the surgery. Objective end-points to body weight change, mobility were monitored cautiously for the first week post-surgery. If required, supplemental analgesia (buprenorphine) would be used.

2.4. Experimental design, data acquisition and cardiovascular monitoring

A range of pharmacological agents either negative (*i.e.* saline (IV/SC) or water (PO)), or positive (remifentanil, flecainide, dopamine, pimobendan, morphine, amrinone, atenolol, and itraconazole) control agents was selected to characterize the rat telemetry model (Table 1). All treatments were administered precisely at the same time of the day with at least 3 days of wash-out between doses. The staff was not allowed to enter the animal room during data acquisition. A negative control (saline or water treatment) using the same dose volume and administration route was administered 2 days prior to positive control administration for within-time comparison with all drugs. Positive chronotropic and inotropic drugs were selected to induce a battery of ECG and hemodynamic changes, and for several drugs, different doses were used for testing potential dose-effect (Table 1). Intravenous injections and infusions were performed using remote dosing from outside of the cage with a permanent catheter to avoid artifacts due to handling stress. Cardiovascular function including systemic arterial blood pressure (diastolic, mean and systolic SABP), left ventricular pressure (systolic, end diastolic LVP, dP/dt + and contractility index) ECG (HR, intervals PR, QRS and QT), BT and locomotor activity were monitored using the radiotelemetry data acquisition program Dataquest ART (Version 4.39, DSI). Contractility index is defined as dP/dt + divided by the pressure at that point. ECG analysis was conducted using semi-automated methods by a single reader to minimize variability (Authier et al., 2010). Cardiovascular parameters were recorded continuously for a period of at least 1 h pre-dosing and for at least 24 h post-dosing every 5 s for the duration of the recording. Data were analyzed and presented using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). Marginal distribution curves using beat-to-beat contractility

Table 1
Cardiovascular positive and negative control drugs.

Drug	Method of administration	Dose level* (mg/kg)
Saline–water	IV/SC–PO	0
Remifentanil	IV	0.03
Flecainide	IV	16
Dopamine	IV	0.1
Pimobendan	PO	3, 10, 30
Morphine	SC	2, 6, 20
Amrinone	PO	10, 30, 100
Atenolol	PO	1, 3, 10, 100
Itraconazole	PO	10, 30, 100

Administration route: PO, oral; SC, subcutaneous; IV, intravenous (*via* tail vein).
* Dose selection was based on data previously obtained in conscious rats that were monitored using telemetry and the scientific literature available.