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Original article

Cardiovascular and respiratory safety pharmacology in Göttingen minipigs: Pharmacological characterization

S. Authier [a,b,*], J. Gervais [a,b], S. Fournier [a], D. Gauvin [b], S. Maghezzi [a], E. Troncy [b]

[a] LAB Research North America, 445 Armand Frappier, Laval, Quebec, Canada H7V 4B3
[b] GREPAQ, Department of Veterinary Biomedicine, Faculté de médecine vétérinaire, Université de Montréal, P.O. box 5000, St-Hyacinthe, Quebec, Canada J2S 7C6

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ABSTRACT

Introduction: Similarities between pigs and humans support the relevance of Göttingen minipigs for regulatory safety pharmacology. The minipig is the species of choice for cardiovascular safety pharmacology when pivotal repeat toxicology studies are conducted in this species. **Methods:** 4 male Göttingen minipigs with cardiovascular telemetry transmitters received intravenous saline, esmolol (0.5, 1, 2, 4 and 8 mg/kg), medetomidine (0.04 mg/kg), remifentanil (0.5, 1, 2, 4, 8 and 16 μg/kg) and dopamine (2, 8, 10, 20, 30 and 50 μg/kg/min) and oral sotalol (3 and 10 mg/kg). Respiratory monitoring was conducted in 3 male and 3 female Göttingen minipigs receiving intravenous saline and methacholine (0, 3.4, 13.5 and 68 μg/kg). **Results:** Heart rate (HR) corrected QT was optimal with a method based on analysis of covariance (QTca) followed by Fridericia's standard formula. Esmolol induced a decrease in HR. Medetomidine was associated with an initial hypertension with bradycardia followed by sustained hypotension, bradycadia and prolonged QTc. Remifentanil induced a dose-dependent QTc shortening with an increase in arterial pressures. Sotalol caused a decrease in HR and systolic arterial pressure with an increase in PR and QTc intervals. Dopamine induced an increase in arterial and pulse pressures. Methacholine increased tidal volume, respiratory rate and minute volume. **Discussion:** The results suggest that the minipig is a valid alternative to other non-rodent species for cardiovascular and respiratory safety pharmacology studies when this species is justified.

1. Introduction

The minipig has gained increased acceptance as an alternative for large animal toxicology and safety pharmacology assessments (van der Laan et al., 2010). The regulatory guideline on non-clinical evaluation of the potential for delayed ventricular repolarization (ICH, 2005) includes swine as a potential species for *in vivo* electrophysiology studies. As for other species, proper justification is required to select the minipig as a non-rodent model for core battery safety pharmacology (ICH, 2000). Absorption, distribution, metabolism and excretion (ADME), presence/homology of drug target in the animal species relative to humans, route of administration (*e.g.* dermal), historical data, species sensitivity to toxicological effects, reproducibility and clinical relevance are common considerations in the species selection process. From an ethical perspective, a case-by-case analysis is recommended to determine applications where the use of minipigs should be favored over other non-rodent animal species (Forster, Bode, Ellegaard, van der Laan, & Steering Group of the RETHINK Project, 2010).

Low variability was previously reported for cardiovascular parameters in telemetered Göttingen minipigs (Stubhan et al., 2008) and supports its use for safety pharmacology. The mRNA and profile expressions of major cardiac ion channel proteins in both atria and ventricle of minipigs were reported to be similar to humans (Laursen, Olesen, Grunnet, Mow, & Jespersen, 2011). Minor ion channel differences were identified between pigs and humans as the 4-aminopyridine- (4-AP-) sensitive transient outward K current (I_{to1}) is not expressed in pigs (Li et al., 2003). Greater anatomical similarities are noted between the porcine and the human heart (Douglas, 1972; Hughes, 1986) compared to dogs (Kato et al., 1987). Although, pigs would not identify QT widening caused by specific I_{to1} blockade, it is generally considered an acceptable species for cardiac safety pharmacology (Pugsley, Authier, & Curtis, 2008).

Validation and pharmacological characterization are central to the acceptance of non-clinical models. Recognized hERG blockers (moxifloxacin, haloperidol), competitive β-adrenergic receptor blocker (sotalol) and a non-selective beta-adrenoreceptor antagonist (propranolol) were shown to induce expected cardiovascular effects in the minipig model (Kano et al., 2005; Markert et al., 2009). While a variety of cardio-active agents have been used in safety pharmacology models using canines (Chaves et al., 2007; Chui et al., 2009; Moscardo,

* Corresponding author. LAB Research Inc., 445 Armand Frappier, Laval, Quebec, Canada H7V 4B3. Tel.: +1 450 973 2240x1403; fax: +1 450 973 2259.
E-mail address: authiers@labresearch.com (S. Authier).

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Fasdelli, Giarola, Tontodonati, & Dorigatti, 2009; Ollerstam et al., 2007) and non human primates (Ando et al., 2005; Authier, Tanguay, Gauvin, Fruscia, & Troncy, 2007; Moscardo, McPhie, Fasdelli, Dorigatti, & Meecham, 2010), limited data remains available from telemetered minipigs. Similarly, a paucity of information is available for respiratory safety pharmacology in minipigs (Bode et al., 2010). We report cardiovascular and respiratory responses of Göttingen minipigs to vehicle-control and various positive control agents in the context of safety pharmacological investigations.

2. Materials and methods

2.1. Statement on use and care of animals

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

2.2. Animal housing

The animal room environment was controlled (temperature 21 ± 3 °C, relative humidity 30–70%, 12 h light, 12 h dark, 10–15 air changes per hour) and temperature and relative humidity were monitored continuously. A standard certified commercial swine chow (Certified Miniswine Diet 7037C™, Harlan Teklad, Madison, WI, USA) was available to each minipig twice daily. Four (4) animals (all males) were assigned to cardiovascular monitoring while six (6) animals (3 males and 3 females) were assigned to respiratory monitoring.

2.3. Animal preparation: telemetry for cardiovascular monitoring and intravenous catheter

Four (4) male Göttingen (*Sus scrofa*) minipigs (ages: 7 months, wt.: 13.0–14.9 kg) were surgically prepared with telemetry transmitters (TL11M3-D70-PCT, DSI, St-Paul, MN, USA). Surface ECG was obtained from all animals prior to surgery to ensure all animals presented normal cardiac conduction. Prophylactic antibiotics (cefazolin 25 mg/kg; Sandoz, QC, Canada) were administered by intramuscular (IM) injection at least 30 min prior to surgery and every 4–8 h for at least 24 h post surgery. Preemptive analgesia (buprenorphine, Temgesic™, 0.05 mg/kg, Schering-Plough, Welwyn Garden City, Hertfordshire, UK) was administered by IM injection before surgery and every 6 to 12 h for at least 3 days post-surgery. Animals were placed on a heating pad and inhaled a mixture of oxygen (O_2) and isoflurane (AErrane™, Baxter Corporation, Mississauga, ON, CAN) with the vaporizer set at 2.0%. Spontaneous breathing was used. During anesthesia, monitoring included heart rate and pulsatile hemoglobin saturation in O_2 (VetOx 4404™ pulse oximeter, Heska, Fribourg, Switzerland). An abdominal midline skin incision was initially made followed by a longitudinal incision (length of approximately 4 cm) in the middle of the *rectus abdominis* muscle (parallel to muscle fibers). The telemetry transmitter was placed on the left side between the *internal abdominal oblique* muscle and the aponeurosis of the *transversus abdominis* muscle. The negative ECG electrode (Data Science International, St-Paul, MN, USA) was tunnelled subcutaneously to a small skin incision at the level of the right thoracic inlet. A small loop (approx. 8 mm diameter) was created with the naked wire from both electrodes and the negative electrode was sutured deep to the muscles. Once the negative electrode was in place, the positive electrode was apposed at the skin surface on the left lateral aspect of the thorax to locate the position of highest QRS amplitude. Saline was used topically to maximize skin conduction when needed. A small skin incision was made at the point of highest amplitude and the electrode was tunnelled and secured to the muscle using non-absorbable sutures (Polybutester 4-0, Novafil™, Tyco Healthcare Group LP, Norwalk, CT, USA). Simultaneous ECG monitoring with the telemetry system was used to evaluate ECG morphology. The arterial pressure line was inserted in the right femoral artery and advanced to the abdominal aorta. A permanent intravenous infusion catheter was inserted in the right femoral vein and was tunnelled to the interscapular region. Surgical sites were rinsed with warm sterile saline and sutured in anatomical planes. Skin incisions were closed using absorbable buried sutures. Permanent IV catheters were maintained on continuous infusion of saline (4.0 mL/h) pending dosing.

2.4. Cardiovascular monitoring

Cardiovascular function monitoring included arterial blood pressure (diastolic, mean and systolic), electrocardiogram, body temperature and physical activity (DataQuest ART Version 3.1, DSI, St-Paul, MN, USA). ECG analysis was conducted using semi-automated methods by a single reader to minimize variability (Authier, Pugsley, Troncy, & Curtis, 2010). Positive control drugs were selected to induce a battery of ECG and hemodynamic changes (Table 1). For saline (Baxter, ON, Canada), medetomidine (Domitor®, Novartis, Mississauga, ON, Canada) and sotalol (Sigma-Aldrich, ON, Canada), cardiovascular parameters were recorded continuously for a period of at least 2 h before dosing and for at least 24 h post-dosing. For esmolol (Brevibloc®, Baxter Corporation, Mississauga, ON, Canada), remifentanil (Ultiva®, Abbott Laboratories Ltd., Vaughan, ON, Canada) and dopamine (Inotropin™, Bristol-Myers Squibb, Montreal, QC, Canada), animals were continuously monitored for a period of at least 2 h before dosing and continuously until at least 10 hpost-dosing. Intravenous injections and infusions were performed from outside of the animal cage with a permanent catheter to avoid artefacts due to handling stress.

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
	4.0
	8.0
IV infusions (duration)	**Dose rate (mg/kg/min)**
Dopamine (30 min step-infusion)	0.002
	0.008
	0.01
	0.02
	0.03
	0.05
Oral administration (PO)	**Dose level (mg/kg)**
Sotalol	3.0
	10