Original article

Cardiovascular and respiratory safety pharmacology in Göttingen minipigs: Pharmacological characterization

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A R T I C L E  I N F O

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A B S T R A C T

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 toxicity 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 Fredericia’s standard formula. Esmolol induced a decrease in HR. Medetomidine was associated with an initial hypertension with bradycardia followed by sustained hypotension, bradycardia 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.

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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, 2005; Hughes, 1986) compared to dogs (Kato et al., 1987). Although, pigs are noted between the porcine and the human heart (Douglas, 1972; 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 β1 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-adrenoceptor 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, 2001; van der Laan et al., 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 protein 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 (Ito) 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).

It is generally considered an acceptable species for cardiovascular safety pharmacology (Pugsley, Authier, & Curtis, 2008).

Bode, Ellegaard, van der Laan, & Steering Group of the RETHINK Project, 2010).

Fasdelli, Girola, 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 7037TM, 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-PECT, DSI, StPaul, 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 injection at least 24 h before dosing and for at least 24 h post-dosing. For esmolol (IV bolus) (Brevibloc®, Baxter Corporation, 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 (Innotrop™, 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 h post-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>
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<th>Table 1 Cardiovascular positive control drugs.</th>
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<td>Dopamine (30 min step-infusion)</td>
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<td>Oral administration (PO)</td>
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2.5. Respiratory monitoring

Respiratory monitoring was performed using a computerized system composed of a data acquisition controller (DAC 8, Scientific Respiratory Equipment Quebec Inc. (SCIReq), Montreal, QC, Canada) connected to a computer (OptiPlex GX280 Workstation, DELL, Dallas, TX, U.S.A.) where a respiratory monitoring software (flexiWare 5.1, SCIReq Inc., Montreal, QC, Canada) was installed. The respiratory hardware connected to the flexiWare 5.1® software included a pneumotachometer (Model 3719 with a 100 L/min capacity, HANS RUDOLPH, MO, U.S.A.) with individual heater controllers (HANS RUDOLPH, MO, U.S.A.). Precision differential pressure transducers connected to the data acquisition matrix were connected with the pneumotachometer (Model UT-PDP-02, 0.2 kPa nominal, SCIReq Inc., Montreal, QC, Canada). Saline (Baxter, ON, Canada) and methacholine (Sigma-Aldrich, ON, Canada) were administered in a Latin square design to each animal (0, 3.4, 13.5 and 68 μg/kg). Respiratory monitoring included respiratory rate (RR), tidal volume (TV) and minute volume (MV) averaged in 5-minute intervals. Animals were acclimated to the respiratory monitoring system prior to dosing.

2.6. Statistical methods

Drug-induced effects were evaluated using an ANOVA for repeated measures (SAS Version 9.1, Cary, North Carolina, USA). A posteriori contrasts were conducted using Dunnett’s test. Statistical tests were performed at the 0.05 threshold of significance. Mean ± SEM data are presented.

3. Results

3.1. Cardiovascular monitoring

Slopes of linear regression (Tattersall, Dymond, Hammond, & Valentin, 2006) for selected QT correction formulas as a function of heart rate were −0.541 ± 0.062 (QTcV; Van de Water, Verheyen, Xhonneux, & Reneman, 1989), −0.183 ± 0.048 (QTcF; Fridericia, 1920), 0.367 ± 0.043 (QTcB; Bazett, 1920) and 0.001 ± 0.006 (QTca; Spence, Soper, Hoe, & Coleman, 1998) ms/bpm.

3.1.1. Esmolol

A decrease in heart rate compared to baseline (103 ± 4 bpm) was noted after esmolol IV bolus administration (Fig. 1) while systemic arterial pressure remained unchanged. The effect on heart rate resolved within 30 min post-administration. No other parameter was significantly altered.

3.1.2. Medetomidine

Medetomidine administered by IV bolus was associated with an initial increase in arterial pressures (systolic, mean and diastolic) combined with a decrease in heart rate (Fig. 2). This hypertensive phase resolved within 7 min after administration and was followed by a prolonged period of relative hypotension and bradycardia. A prolongation of QTcF was observed from 7 min until 6 h post-dose reaching a maximum (+27.1%) at 105 min post-dose. Body temperature was decreased reaching a nadir of −7.7% (compared to baseline) at 3 h post-dose.

3.1.3. Remifentanil

A dose-dependent increase in arterial blood pressures (Fig. 3) with a decrease in QTc (Fig. 4) was noted following remifentanil IV bolus. At high dose (16 μg/kg), peak increases in systolic (+ 76 mm Hg; +54%) and diastolic (+ 58 mm Hg; +61%) pressures were noted immediately after dosing. The effect on QTc (shortening) reached statistical significance at all doses from 1 to 16 μg/kg. Variations in heart rate were noted without statistically significant differences.

3.1.4. Sotalol

Oral administration of sotalol was associated with a decrease in heart rate (3 and 10 mg/kg) and systolic arterial pressure (10 mg/kg) combined with an increase in PR interval (3 mg/kg) and QTcF (10 mg/kg) (Fig. 5). The increase in QTcF was intermittently significant from 3 h 30 min until 9 h post-dose.

3.1.5. Dopamine

Dopamine infusion resulted in an increase in arterial pulse pressure at doses of 0.008, 0.01, 0.02, 0.03 and 0.05 mg/kg/min (Fig. 6). At high dose (0.05 mg/kg/min), a significant increase in systolic arterial pressure (15 min post-infusion start) and heart rate (+66.7% at infusion completion) were also observed (p<0.05).

3.2. Respiratory monitoring

Respiratory minute volume per kilogram in Göttingens minipigs was comparable to other non-rodent laboratory animal species (Fig. 7). Methacholine at high dose (68 μg/kg) induced a transient increase in respiratory rate (Fig. 8), tidal volume (Fig. 9) and minute volume (Fig. 10) with recovery within 15 min post-administration.

4. Discussion

The use of minipigs in pivotal regulatory toxicology studies has increased (Jacobs, 2006) and corresponding cardiovascular safety pharmacology studies are typically conducted in the same species. The RETHINK project offers a complete and comprehensive review of current minipig use in regulatory toxicology assessments of new chemical entities and biologics (Curtis, 2010; Forster et al., 2010). The minipig is the non-rodent species of choice for drug candidate intended for dermal application and the classical dosing paradigm using a Latin square design can be used with telemetered animals. When only one dose level is selected, a cross-over design between vehicle/control and treatment is typically used. A skin surface of up to 10% of body surface area (BSA) considered the maximal practical area can be selected for dosing. Formula to calculate BSA in minipigs can be used to estimate the surface dosing area: Area (cm²) = 700 * Weight in kg^(3/4) (Maynard, Loosli, Hintz, & Warner, 1979). A minipig of 10 kg has an estimated BSA of 3936 cm² and a practical dosing surface of 20 cm x 20 cm can be permanently delineated using tattooed dots under anesthesia or a permanent skin marker. The dorsal area is preferred to facilitate application and is amendable to an occluded or semi-occluded dressing to protect the dosing site when deemed necessary.
required. Cardiovascular safety pharmacology studies do not typically include toxicokinetic assessments. When conducting cardiovascular safety pharmacology studies with dermal application, quantification of systemic exposure may be valuable for cardiovascular result interpretation. The relevance of cardiovascular safety pharmacology studies depends on achieving systemic exposure pertinent to the clinical situation. Physical restraining which is required for blood collections would generate significant cardiovascular artefacts in all species including minipigs. Permanent intravenous femoral catheters with jacket and tether for remote collection from outside of the animal room can offer a practical alternative to quantify systemic exposure without interferences with cardiovascular monitoring when toxicokinetic is considered scientifically justified. This experimental set-up requires continuous IV infusion with saline (e.g. 6.0 mL/h) between collection time points to maintain patency.

As previously reported in Göttingen minipigs, individual QT correction (QTca) provided optimal results with minimal changes to QTc at different heart rates (Stubhan et al., 2008). Consistent with previous reports (Kano et al., 2005), Fridericia’s formula provided the most stable standard correction for QT with heart rate changes.

Esmolol is a short acting cardioselective beta1 blocker expected to have negative chronotropic effects (Berne & Levy, 1992). Intravenous administration of esmolol to telemetered non human primates was associated with significant decreases in heart rate and arterial pressures (Authier et al., 2007). The minipig showed a significant decrease in heart rate but no effect on systemic arterial pressures. Compensatory mechanisms such as peripheral vasoconstriction could explain the lack of effects on systemic arterial pressure in the minipig despite negative chronotropic effects but in-depth cardiovascular investigations would be needed to confirm this hypothesis.

Medetomidine is a selective alphax2-adrenergic agonist recognized to induce an initial phase of hypertension followed by hypotension and bradycardia in dogs (Pyppendop & Verstegen, 1998), non human primates (Capuano, Lerche, & Valverde, 1999) and humans (Penttila, Helminen, Anttila, Hinkka, & Scheinin, 2004). The degree of bradycardia observed in Göttingen minipig was comparable to dogs (Vainio & Palmu, 1989) and non-human primates (Capuano et al., 1999). As observed in other animal species (Authier et al., 2007;
Kinjavedkar et al., 1999), medetomidine also induced a significant increase in QTc.

Remifentanil is a short-acting (μ)-opioid agonist. Transient increases in arterial pressures and heart rate were observed after remifentanil administration to awakenn humans (Glass et al., 1993). Similar effects were observed in non-human primates (Authier et al., 2007). A predominant dose-dependent increase in arterial pressures was observed in the minipigs without significant changes in heart rate. The severity of the effects on arterial pressures (+54% for systolic and +61% for diastolic) would be expected to induce reflex bradycardia through baroreceptor activation (Buckley et al., 1979).

The paradoxical absence of heart rate effects (non-significant +13% for heart rate) suggests that remifentanil may have induced some positive chronotropic activity, as observed in other species, with attenuation of the response to baroreceptor activation. A clinical dose (1.0 μg/kg followed by an infusion of 0.5 μg/kg/min) of remifentanil administered intravenously to anesthetized pigs did not induce any effect on QTc (Zaballlos et al., 2009). Our results in Göttingen minipigs suggest a dose-dependent shortening of QTc. An attenuation of QT prolongation associated with tracheal intubation during anesthesia induction in humans was observed with concomitant administration of remifentanil (Kim & Chang, 2011; Kweon et al., 2008). Significant decreases in QT dispersion (QTD) and QTc were recently reported (Cafero, Di Minno, & Di Iorio, 2011) with remifentanil in humans undergoing anesthesia induction. Possible mechanisms for reduction in QT prolongation during induction of anesthesia and tracheal intubation include activation of the autonomic system (Lindgren et al., 1993). Fluctuations in autonomic tone have been associated with variations in QT interval duration at the same heart rate (Soloviev, Hamlin, Barrett, Chengelis, & Saefer, 2006) and the attenuation of QT prolongation with remifentanil could be, at least partially, mediated through autonomic modulations.

Kano et al. (2005) have reported that oral administration of sotalol to minipigs at the same dose level (10 mg/kg) was associated with a significant increase in PR and QTcF between 0.5 and 4 h after dosing without significant effects on arterial pressure (~3% to ~7%). We observed similar effects on PR interval and QTc but also noted a decrease in heart rate (3 and 10 mg/kg) and systolic arterial pressure at 10 mg/kg. In healthy volunteers, sotalol induced an increase in PR interval, a decrease in heart rate (Kantelip, Trolese, Cadilhac, Pechadre, & Duchêne-Marullaz, 1986), a QTc prolongation (Barbey et al., 1999) and a decrease cardiac index (Thumala et al., 1971). Sotalol was associated with negative chronotropic and inotropic effects in healthy volunteers but did not induce any vasodilatory effects in non-human primates (Authier et al., 2008) and a decrease cardiac index (Thumala et al., 1971). Sotalol (0.018 mg/kg) produce pressor responses and increase in arterial pressures (+54% for systolic and +61% for diastolic) would be expected to induce reflex bradycardia through baroreceptor activation (Buckley et al., 1979).

In summary, The RETHINK project has highlighted the need to expand the safety pharmacology data with minipigs available in the public domain for proper deployment of the model (Bode et al., 2010). Our results further support the value of the Göttingen minipig for cardiovascular and respiratory safety pharmacology investigations. This species is considered a valuable alternative to traditional non-rodent species for regulatory safety pharmacology.

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References


