The Fentanyl/Etomidate-Anaesthetised Göttingen (FEAG) Minipig: A Cardiovascular Safety Model

ABSTRACT
This paper describes the development of a non-rodent in vivo cardiovascular safety model: the fentanyl/etomidate-anaesthetised Göttingen (FEAG) minipig model. There is minimal influence from the applied anaesthetic regime on the baseline cardiovascular, pulmonary and haematological parameters within this model. As shown in the results, all measured parameters were comparable to published conscious Göttingen minipig data. Hemodynamic, cardiac, electrophysiological, respiratory and arterial blood parameters were relatively stable for at least three hours, and the QT interval could be corrected for changes in heart rate using a linear formula: QTc = QT – 1.6 (60 – HR). Intravenous infusion of dofetilide induced a significant QTc prolongation of +71 ms (+18%). Further characterisation of the FEAG minipig model is ongoing and will be published soon.

INTRODUCTION
To date, miniature pigs have been used extensively in cardiovascular research, because both the hemodynamics and electrophysiology of these animals appear to be similar to humans. Consequently, miniature pigs have become the species of choice for evaluating the safety of some new molecular entities (NME’s). In cardiovascular safety studies, Göttingen minipigs have mainly been used in conscious animal settings (i.e. static condition in a sling or ad libitum), and less frequently in anaesthetised models. The big advantage of the latter is the freedom to push the doses/exposures way beyond levels where CNS, respiratory or GI side effects are likely to occur. Additional advantages of anaesthetised models include the ability to perform measurements of many different electrophysiological, respiratory, and arterial blood parameters that are not feasible to obtain in conscious minipigs. Moreover, it is difficult to evaluate in anaesthetised animals, we hereafter propose a FEAG-specific correction formula. Finally, in this paper, we illustrate the QT-sensitivity of the FEAG minipig to a known and broadly used I duty blocker (dofetilide).

MATERIAL AND METHODS
1. Animals
All published experiments have been conducted in accordance with “The provision of the European Convention” on the protection of vertebrate animals which are used for experimental and other scientific purposes, and with “the Appendices A and B”, made at Strasbourg on 18 March 1986 (Belgian Act of 18 October 1991) and the Commission recommendation of 18 June 2007 on guidelines for the accommodation and care of animals used for experimental and other scientific purposes (2007/526/EC). In all the experiments, male Ellegaard Göttingen minipigs aged 18 to 24 months, with a body weight ranging from 27.7 to 39.5 kg, were used. The minipigs were housed on bedding (wood shavings) and in pairs in an AAALAC-accredited facility, with a controlled room temperature (18–24 °C) and a day/night cycle of 12 h light/12 h dark. The animals had access to water ad libitum, were fed between 7:00 and 8:00 AM with a standard minipig diet (sniff® MPig-H) and were allowed to stay in an outdoor playground twice a week to socialise. The animals were easy to handle and underwent routine clinical examination. The animals were found to be healthy and active before use. Food (but not water) was withheld for at least 12 hours prior to anaesthesia and experimentation.

2. Anaesthetic regime
The minipigs were premedicated with an intramuscular injection of 3 mg/kg of azaperone (Stresnil®, Janssen Animal Health, Beersel, Belgium) behind the ear at the animal house before transportation. In addition, an intramuscular injec-
Set-up for the Fentanyl/Etidomate anaesthetisation of Göttingen (FEAG)

**Experimental design**

To investigate the relationship between the duration of the QT interval and heart rate, we used six minipigs with high basal heart rates. During the stabilisation period (1–2 hours) the animals received bolus injections of saline with 5% dextran and 2.5% glucose to lower the heart rate. The QT/RR and QT/HR data of these minipigs were plotted and linear correlation variables were calculated. To evaluate the baseline values and the stability of all parameters, six minipigs were administered with six volumes (0.032, 0.063, 0.125, 0.25, 0.5 and 1 ml/kg i.v.) of saline, infused over 5 minutes at 30 minute intervals, and all parameters were measured or calculated at selected times (pre-dose and at the end of each infusion). In four of these saline-treated minipigs, dobutamine (0.05 mg/kg i.v.) was infused over 10 minutes at the end of the experiment.

**RESULTS AND DISCUSSION**

1. **QT correction**

To evaluate the relationship between the duration of the QT interval and changes in heart rate (HR), the heart rate values of six minipigs (all male) with a wide range in heart rates (and RR intervals) during the stabilisation period were plotted against the uncorrected QT intervals. This resulted in a non-linear relation between RR and QT, comparable with data from telemetered minipigs. However, a linear relationship was noted after plotting HR against QT data, with comparable slopes and correlation coefficients (R^2) for all animals (Figure 1).

These data (Table I) were used to calculate a mean slope and the following formula was derived:

\[ \text{QTC} = \text{QT} - 1.6 \times (60 - \text{HR}) \]

To evaluate our proposed formula, three QTC values calculated according to commonly used formulas ( Bazett, Fridericia and真心) were compared with the values calculated from our formula.
Fridenica and Van De Water) were plotted against HR (Figure 2). Linear regression lines are displayed in the graphs and correlation coefficients (R²) and slopes are shown in the graphs; the most effective formula will show the lowest R² and a slope close to zero. As described by others,[8] the Bazett formula shows an over-correction, resulting in a positive slope of the regression line. The other two (Fridenica and Van De Water) showed both an under-correction (slopes were -0.4748 and -0.976, respectively).

However, our proposed correction formula showed a horizontal regression line (slope = 0.0373), and no correlation between heart rate and QTc (R² = 0.004). We realise that this formula is constructed on the basis of a small group of six animals and that more experiments will be necessary to prove the utility of this formula. In our vehicle group, a decrease of heart rate (-24 b.p.m.) after 3 hours resulted in an increase of QT (+33 ms) and was totally corrected by this formula, but the value of this formula can only be confirmed by using it in further studies in our own laboratory and other laboratories.

2. Baseline values and stability
Baseline values (mean ± SEM) of all measured and calculated parameters and the stability of these parameters (±3% after i.v. saline administration from 0.032 to 1 ml/kg; duration of 3 hours) are listed in Table 2. Not all parameters were measured in all minipigs due to technical reasons. For example, cardiac output (CO), stroke volume (SV), systemic vascular resistance (SVR) and pulmonary vascular resistance (PVR) were measured in only 2 animals and must be regarded as provisional. Most parameters were stable over a period of 3 hours and changed less than 10%. Heart rate (HR), pressure rate product (P;P) and left ventricular contraction (LV dp/dt max) showed slight to minor decreases (-30%, -24%, -18% and -13%, respectively), and left ventricular end diastolic pressure (LVEDP) showed an increase (+21%) during the saline experiments (+2 ml/kg i.v.) in this study. The HR decrease was accompanied, as expected, by an increase in the duration of the RR, PQ and QT intervals. The QT and QTc intervals were relatively long, compared to values obtained by others[9 and 10] this was probably caused by the mild hypokalaemia in addition to the low body temperature.[10] The increase in the left-right dispersion

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Table 1: Heart rate (HR) and QT interval (QT) ranges, linear correlation coefficients (R²) and slopes of six anaesthetised male minipigs (data obtained during stabilization period).

Figure 1: QT (ms) versus RR (ms) - plot (A) and QT (ms) versus HR (b.p.m) - plot (B); data derived from six anaesthetised male Göttingen minipigs (418 data points).

Figure 2: Corrected QT (QT in ms) versus Heart Rate (HR in beats per minute); a comparison of different QT correction formulae (Bazett, Fridericia, Van De Water and the FEAG minipig-specific formula).
The fentanyl/etomidate-anesthetised Göttingen (FEAG) minipig is a potential non-rodent, in vivo model that can be used in safety pharmacology. Indeed, a wide range of hemodynamic, cardiac electrophysiological, respiratory and arterial blood parameters can be measured, have baseline values within a physiological range, and remain stable for a relatively long period. A formula to correct the duration of the QT interval for changes in heart rate is proposed. As expected, dofetilide was found to prolong the QT and QTc intervals.

**CONCLUSION**

The fentanyl/etomidate-anesthetised Göttingen (FEAG) minipig is a potential non-rodent, in vivo model that can be used in safety pharmacology. Indeed, a wide range of hemodynamic, cardiac electrophysiological, respiratory and arterial blood parameters can be measured, have baseline values within a physiological range, and remain stable for a relatively long period. A formula to correct the duration of the QT interval for changes in heart rate is proposed. As expected, dofetilide was found to prolong the QT and QTc intervals.

**REFERENCES**


