**SUPPLEMENTAL MATERIAL**

**Supplemental Methods and Results**

***Safety study***

The safety of the compounds was assessed by clinically observing all horses for sweating, excitement, muscle shivering or weakness, as well as assessing the levels of cardiac biomarkers, measuring blood pressure throughout drug administration in the safety study, and analysing QRS duration and QTc on ECGs.

*Drug treatment*

All horses were catheterised in the jugular vein with a 12 G intravenous catheter (Intraflon 2, Vycon, Wiltshire, United Kingdom), which was used for drug infusion and blood sampling. The order of procedures was randomised. Dofetilide was given at a dose rate of 8.0 µg/kg BW, and ranolazine was given at a dose rate of 2.4 mg/kg BW, while saline was administered at 0.2 ml/kg BW. All solutions were administered at a rate of 0.0125 ml/kg BW/min. The drugs were administered in two stages, in case of a severe adverse reaction: firstly, 1/3 of the total dose was administered and 20 min after the end of this infusion, the remaining 2/3 of the dose was administered.

*Blood samples for analysis of cardiac Troponin I and Creatinine Kinase fraction MB*

Blood samples from all horses included in the safety study were collected at three time points: before drug infusion, and at 4 hours and 24 hours after the last drug infusion. In the electrophysiology study, blood was sampled in six horses (ID4-ID9) prior to drug infusion, and at 4 hours and 24 hours after the induction of AF. Blood was sampled for analysis of cardiac Troponin I (cTnI) and Creatinine Kinase fraction MB (CK-MB). Samples were collected in lithium heparin tubes (BD A/S, Albertslund, Denmark) and stored on ice for a maximum of 7 hours before centrifugation at 43 RPM for 10 min. Following this, 3 ml of plasma were transferred to cryovials and frozen at ‑80°C until further analysis. cTnI was tested by a sandwich immuno-analysis using direct chemiluminometric technology, with an assay detection limit of 0.006 ng/ml. CK-MB analysis was performed using a two-centre sandwich immuno-analysis, with an assay detection limit of 0.180 ng/ml.

Data analysis was performed using R 3.3.1 software (The R Foundation for Statistical Computing, Vienna, Austria). Plasma levels of the biomarkers were compared before and after drug administration using a one-way Kruskal-Wallis analysis of variance. The association between the biomarkers and number of burst-pacings was calculated using linear regression.

Levels of cTnI in the blood were not altered by the drug treatments at 4 hours (*P* = 0.32) or 24 hours post-treatment (*P* = 0.33). Similar results were found for levels of CK-MB at 4 hours (*P*= 0.80) and 24 hours (*P* = 0.79) post-treatment. In order to examine the effect of interventions on the heart, we assessed whether repeated pacing of the right atrium resulted in myocardial damage based on the correlation between the levels of cTnI and CK-MB and the number of atrial burst-pacings the horses received. No significant increase was found in the biomarkers within 4 hours (cTnI: *P* = 0.49, CK-MB: *P* = 0.88) or 24 hours (cTnI: *P* = 0.47, CK-MB: *P* = 0.40) of AF induction.

*Blood pressure*

A 20 G arterial cannula (BD A/S, Albertslund, Denmark) was placed in the transverse facial artery and connected to an electronic pressure transducer (Carescape Monitor B650, GE Healthcare, Brøndby, Denmark) to record blood pressure measurements. For non-invasive blood pressure measurement, a cuff was placed around the tail base of the horse. Blood pressure was continually monitored from before until 1.5 hours after the drug infusion, and was recorded for subsequent analysis.

Blood pressure was unchanged following all treatments (see Fig. S.1).

*ECG analysis*

ECGs were obtained throughout each procedure and 24 hours after drug infusion, using a Holter unit (Televet®, Engel Engineering Services GmbH, Heusenstamm, Germany).[1](#_ENREF_1) QT intervals and QRS durations were manually analysed on lead II. The QT measurements were corrected using a piecewise linear regression model.[2](#_ENREF_2" \o "Pedersen, 2013 #3) Data analysis was performed using GraphPad Prism 5 software (GraphPad Software, San Diego, CA, USA).

**Supplemental Tables**

**Table S.1 Overview of animals included in the study**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Horse ID** | **Sex** | **Weight****(kg)** | **Age****(years)** | **Procedure type** | **Procedure order** |
| **ID 1** | Mare | 467 | 8 | Safety | Dof – Ran – Combi – Control |
| **ID 2** | Mare | 543 | 4 | Safety | Combi – Dof – Ran – Control  |
| **ID 3** | Mare | 542 | 11 | Safety | Control – Dof – Combi – Ran  |
| **ID 4** | Mare | 490 | 13 | Electrophysiology | Dof – Ran – Control – Combi |
| **ID 5** | Gelding | 508 | 5 | Electrophysiology | Dof – Ran – Control – Combi  |
| **ID 6** | Mare | 454 | 11 | Electrophysiology  | Control – Dof – Ran – Combi |
| **ID 7** | Gelding | 477 | 10 | Electrophysiology | Control – Dof – Ran – Combi |
| **ID 8** | Gelding | 514 | 4 | Electrophysiology | Control – Ran – Dof – Combi  |
| **ID 9** | Mare | 445 | 7 | Electrophysiology | Dof – Control – Ran – Combi |
| **ID 10** | Mare | 494 | 7 | Electrophysiology | Control – Dof – Ran – Combi |
| **ID 11** | Mare | 530 | 5 | Electrophysiology | Ran – Control – Dof – Combi  |
| **ID 12** | Mare | 555 | 14 | Electrophysiology | Dof – Control – Ran – Combi |
| **Mean ±SD** |  | **502 ±37** | **8.3 ±3.5** |  |  |

Dof = dofetilide; Ran = ranolazine; Combi = combination of dofetilide and ranolazine.

**Table S.2 Time from start of first drug injection to cardioversion**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Horse ID | Saline (min) | Dofetilide (min) | Ranolazine (min) | Combination (min) |
| ID 4 | 1.9 | 62.8 | 17.7 | 21.0 |
| ID 5 | 138.3 | 67.8 | 93.1 | 110.7 |
| ID 6 | 247.6 | 9.8 | 51.3 | 10.3 |
| ID 7 | 136.3 | 52.2 | 9.7 | 6.8 |
| ID 8 | 175.6 | 57.9 | 72.1 | 4.0 |
| ID 9 | 4.6 | 35.9 | 40.1 | 24.8 |
| ID 10 | 166.7 | 31.1 | 46.3 | 4.5 |
| ID 12 | 155.4 | 218.4 | 76.0 | 13.6 |
| Mean ±SEM | **128.3 ±29.9** | **67.0 ±22.7** | **50.8 ±10.2** | **24.5 ±12.6** |

**Supplemental Figures and Figure Legends**

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**Figure S.1 Blood pressure measurements following drug treatment.** Invasive (**A**, **C**, **E**, **G**) and non-invasive (**B**, **D**, **F**, **H**) procedures during the safety study following the administration of saline (**A**, **B**), dofetilide (**C**, **D**), ranolazine (**E**, **F**) and a combination of dofetilide and ranolazine (**G**, **H**). The grey areas represent dose administrations.

**Supplemental References**

1. Haugaard MM, Pehrson S, Carstensen H, Flethoj M, Hesselkilde EZ, Praestegaard KF, et al. Antiarrhythmic and electrophysiologic effects of flecainide on acutely induced atrial fibrillation in healthy horses. J Vet Intern Med2015; 29 (1):339-347.

2. Pedersen PJ, Kanters JK, Buhl R, Klaerke DA. Normal electrocardiographic QT interval in race-fit Standardbred horses at rest and its rate dependence during exercise. J Vet Cardiol2013; 15 (1):23-31.