

# Antiarrhythmic Effects of Combining Dofetilide and Ranolazine in a Model of Acutely Induced Atrial Fibrillation in Horses

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**Background:** Antiarrhythmic compounds against atrial fibrillation (AF) often have reduced efficacy and may display cardiac and/or noncardiac toxicity. Efficacy can be improved by combining 2 compounds with distinct mechanisms, and it may be possible to use lower doses of each compound, thereby reducing the likelihood of adverse side effects. The purpose of this study was to investigate whether the effective doses of dofetilide and ranolazine can be reduced if the drugs are combined.

**Methods:** Dofetilide, ranolazine, and a combination of these were administered in 4 incremental dosing regimens to horses with acutely pacing-induced AF. Time to cardioversion, atrial effective refractory period, and AF vulnerability and duration were assessed.

**Results:** Of 8 horses, 6 cardioverted to sinus rhythm after infusion with a combination of 0.889  $\mu\text{g}/\text{kg}$  dofetilide and 0.104 mg/kg ranolazine. Two horses cardioverted with 0.104 mg/kg ranolazine alone, and 3 cardioverted with 0.889  $\mu\text{g}/\text{kg}$  dofetilide alone. The combination therapy decreased AF vulnerability ( $P < 0.05$ ) and AF duration ( $P < 0.05$ ). No change in atrial effective refractory period was detected with any of the drugs.

**Conclusions:** The combination of dofetilide and ranolazine showed increased antiarrhythmic effects on acutely induced AF in horses, affecting time to cardioversion, AF vulnerability, and AF duration.

**Key Words:** atrial fibrillation, horse, dofetilide, ranolazine, combination therapy

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## INTRODUCTION

Atrial fibrillation (AF) is the most common clinical arrhythmia in both humans and horses.<sup>1</sup> However, current treatment options for restoring sinus rhythm are not optimal because available antiarrhythmic compounds possess cardiovascular and/or noncardiovascular toxicity, and the efficacy is limited.<sup>2</sup> In recent years, ranolazine has been investigated for its antiarrhythmic potential both alone and in combination.<sup>3–5</sup> Ranolazine has previously been reported to suppress QT-related arrhythmias,<sup>6</sup> but a recent study reported an increased risk of torsades de pointes ventricular tachycardia (TdP) associated with its use.<sup>7</sup> Ranolazine blocks a number of ion currents, most notably the late sodium current ( $I_{\text{Na,late}}$ ) and in higher doses, the peak sodium current ( $I_{\text{Na,peak}}$ ) and the rapid activating delayed rectifier potassium current ( $I_{\text{Kr}}$ ) also.<sup>8,9</sup>

Dofetilide is a potent  $I_{\text{Kr}}$  blocker and is used as an antiarrhythmic agent in the treatment of AF.<sup>2</sup> In the DIAMOND study,<sup>10</sup> dofetilide was found to be effective for both cardioversion and maintenance of sinus rhythm in patients with congestive heart failure and AF. However, use of dofetilide may result in TdP because of a prolongation of the QT interval.<sup>11</sup> This QT-prolonging effect is dose-dependent,<sup>2</sup> and by combining the compound with another antiarrhythmic compound, it may be possible to reduce the dose of individual compounds, thus minimizing their adverse effects. Furthermore, the combination of more than one drug is hypothesized to increase the efficacy against AF, and some studies have already addressed this using ranolazine.<sup>4,12,13</sup>

The horse has been advocated as a promising animal model of AF because of the similarity to human physiology, including the cardiac ion channel distribution<sup>14</sup> and occurrence of AF.<sup>1</sup> Furthermore, AF can be easily induced experimentally in horses. Our research group has developed a model of acutely induced AF in horses, which allows us

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to examine the antiarrhythmic effects of different compounds.<sup>14,15</sup>

The present study was designed to test the hypothesis that combining dofetilide with ranolazine will make it possible to reduce the doses of both drugs to subefficacious levels and still reach the same antiarrhythmic potential as when either compound is used alone in higher doses.

### METHODS

The study was approved by the local ethical committee at the Department of Veterinary Clinical Sciences, University of Copenhagen and The Danish Animal Experiments Inspectorate (license number 2012-15-2934-00198) and was performed in accordance with the European Commission Directive 86/609/EEC.

Twelve Standardbred horses were included [body weight (BW) 502 ± 37 kg, age 8.3 ± 3.5 years], of which 3 mares were enrolled in a safety study, and 6 mares and 3 geldings in an electrophysiology study (see **Material Table S1, Supplemental Digital Content 1**, <http://links.lww.com/JCVP/A283>). Before the study, the horses underwent a clinical examination and had a 24-hour electrocardiography (ECG) recording (Televet, Engel Engineering Services GmbH, Heusenstamm, Germany), as well as a routine echocardiographic examination (2-D, M-mode and colour-flow Doppler; Vivid I ultrasound machine with a 1.5 MHz phased array probe, GE

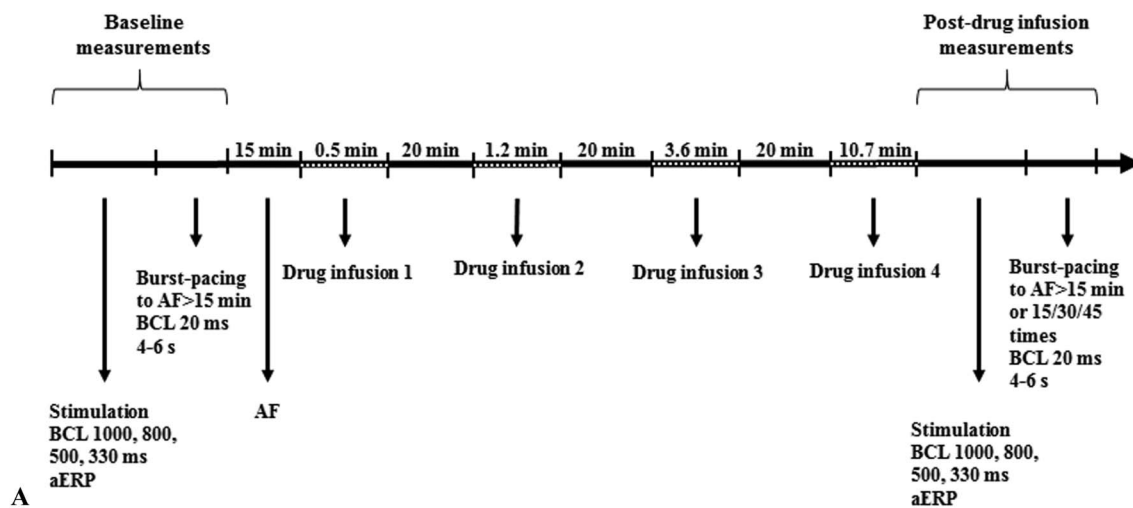
Healthcare, Horten, Norway). Only horses with no signs of cardiovascular disease were included. All horses were included in 4 procedures [saline (control), dofetilide, ranolazine, and the combination of dofetilide and ranolazine], with 7 days recovery period in between. All procedures were performed on nonsedated horses restrained in a stock with access to hay and water. The horses were trained to stand quietly for longer periods before the studies, and therefore, no anesthesia was needed.

### Drugs

Dofetilide (Cat. No. 3757, Tocris Bioscience, R&D systems, Abingdon, United Kingdom) was dissolved in 50% Poly (ethylene glycol) average M<sub>n</sub> 400 (202398 Aldrich, Sigma-Aldrich, MO) and 50% saline (NaCl 9 mg/mL, B. Braun, Melsungen, Germany) to a solution of 0.04 mg/mL. Ranolazine dihydrochloride (Cat. No. 3118, Tocris Bioscience, R&D systems, Abingdon, United Kingdom) was dissolved in saline to a solution of 14 mg/mL. The ranolazine solution was pH-calibrated using 1M NaOH to a pH of just above 5.

### Safety Study

The safety study is described in detail in the **Supplemental Digital Content 1** (see **Table S1**, <http://links.lww.com/JCVP/A283>).



**B**

Treatment	Infusion 1	Infusion 2	Infusion 3	Infusion 4
Saline (ml/kg)	0.008	0.015	0.044	0.133
Dofetilide (µg/kg)	0.296	0.593	1.778	5.333
Ranolazine (mg/kg)	0.104	0.208	0.624	1.872
Dofetilide (µg/kg) Ranolazine (mg/kg)	0.889 0.104	0.208	0.624	1.872

**FIGURE 1.** Experimental protocol. A, Outline of the experimental protocol. B, Schematic overview of the doses used at the different drug infusions.

## Electrophysiology

An overview of the experimental protocol is presented in Figure 1A. Two multipolar steerable nonfixative electrodes (Inquiry Steerable Diagnostic Catheter, 6Fr/110 cm; St. Jude Medical, MN) were used for each horse; 1 for atrial pacing and 1 for obtaining atrial electrograms, as previously described.<sup>15</sup> The electrodes were placed through 2 introducer sheaths (One Piece/Tuohy-Borst Catheter Introducer with Integral Hemostasis Valve, 8F, Argon Medical Devices, Holte, Denmark) in 1 of the jugular veins and advanced to the right atrium using the deflections on the electrogram to determine positioning. The recording electrode was placed so that the deflection on the atrial electrogram appeared in conjunction with the P wave on 2 simultaneously recorded surface ECGs. The pacing electrode was positioned to have consistent atrial capture at 60 beats per minute (bpm), with a threshold of excitation lower than 1.4 mA. Intra-atrial recordings and surface ECGs were monitored and stored for later analysis using Labchart 7 software (ADInstruments, Oxford, United Kingdom).

One horse (ID11) was excluded from the study, as it did not complete the combination procedure because of the development of supraventricular tachycardia during an AF episode before drug administration.

## Atrial Refractory Period

Atrial effective refractory periods (aERPs) were determined to be the longest S1-S2 stimuli interval failing to capture with 9 basic stimuli (S1) followed by an extra

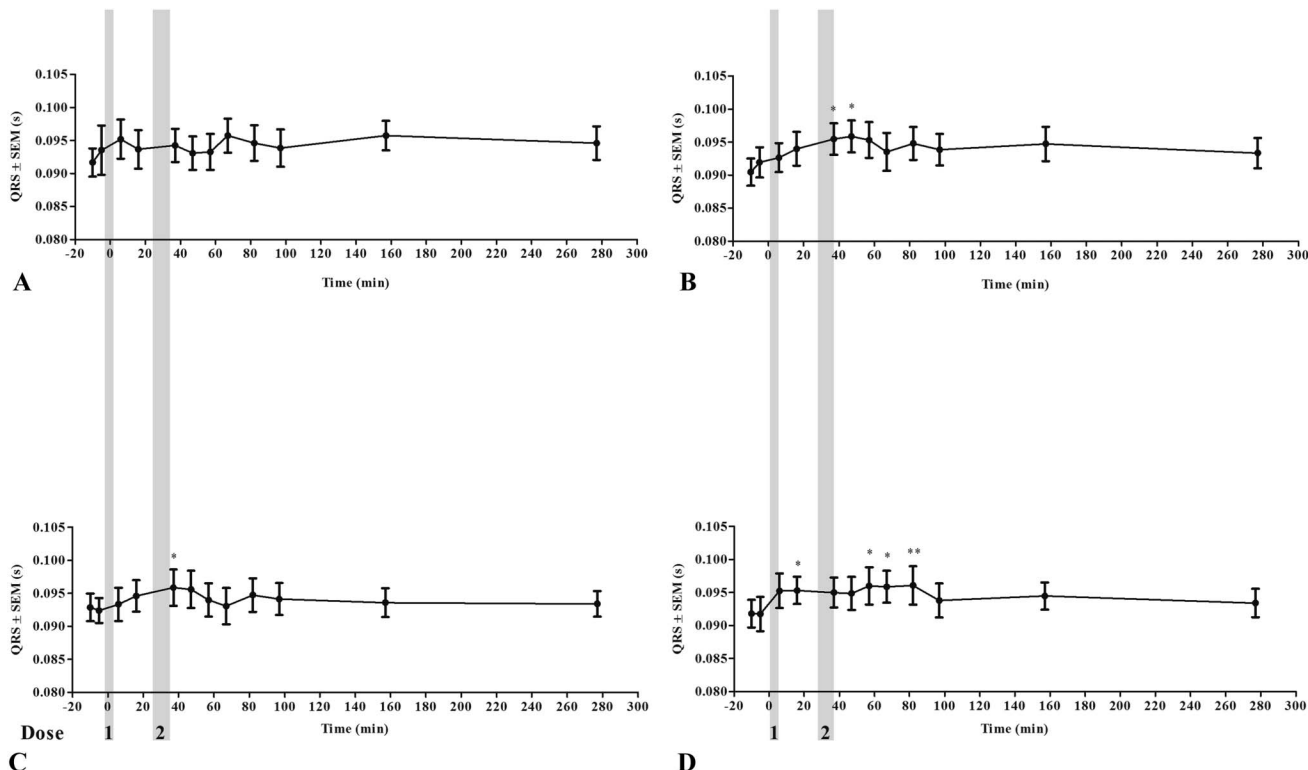
stimulus (S2) with 10-millisecond increments (pulse width 2 milliseconds, current 3 mA). Measurements were performed 3 times and averaged at atrial pacing rates of 60, 75, 120, and 182 bpm before and after drug administration. Each S1-S2 interval was applied a minimum of 3 times at each pacing rate, and aERP was determined as the S1-S2 interval before the one at which complete or intermittent atrial capture occurred.

## AF Induction, AF Vulnerability, and AF Duration

AF was induced after aERP measurements using a current of 10 mA and burst-pacing at 50 Hz for 4–6 seconds. Drug treatment was initiated once the horse developed an AF episode longer than 15 minutes. After drug administration and cardioversion, aERP was measured and an attempt was made to reintroduce AF. After treatment, burst-pacing was performed until AF lasted for more than 15 minutes, or a maximum of 15, 30, or 45 times in cases where the induction of AF before drug administration required  $\leq 15$ ,  $\leq 30$ , and  $\leq 45$  burst-pacings, respectively. The number of burst-pacings required until AF that lasted for more than 15 minutes was counted to evaluate AF vulnerability. The mean duration of AF episodes after burst-pacings (maximum 15 minutes) was termed AF duration.

## Drug Treatment

The order of saline, dofetilide, and ranolazine procedures was randomized, whereas all horses received the



**FIGURE 2.** QRS duration during drug treatment. QRS duration during the 4 safety procedures: saline/control (A), dofetilide (B), ranolazine (C), and a combination of dofetilide and ranolazine (D). The gray areas represent dose administrations. \* $P < 0.05$ ; \*\* $P < 0.01$ .

combination treatment in their last procedure. Dofetilide was administered intravenously at a dose of 8.0 µg/kg BW, in accordance with recommendations in humans.<sup>16</sup> Ranolazine was administered intravenously at a dose of 2.4 mg/kg BW, in accordance with studies in pigs,<sup>17</sup> as these studies have demonstrated antiarrhythmic effects using an intravenous route of administration, whereas studies in humans use oral administration. Saline was administered at 0.2 mL/kg BW to mimic the volume of the drugs infused. All solutions were administered at a rate of 0.0125 mL/kg BW/min according to the recommendations for dofetilide administration.<sup>16,18</sup> The doses were administered in 4 steps—each with a 3-fold increase in the total dose (Fig. 1B). Each administration step was given with 20-minute intervals. For the combination procedure, ranolazine was administered in 4 steps, using the same dose as when administered alone. Dofetilide was given with the first dose of ranolazine at a dose of 0.889 µg/kg, as this corresponded to the last noncardioverting dose of dofetilide when used alone. All horses were given all 4 doses of each drug regardless of cardioversion time, to allow for the comparison of electrophysiology parameters.

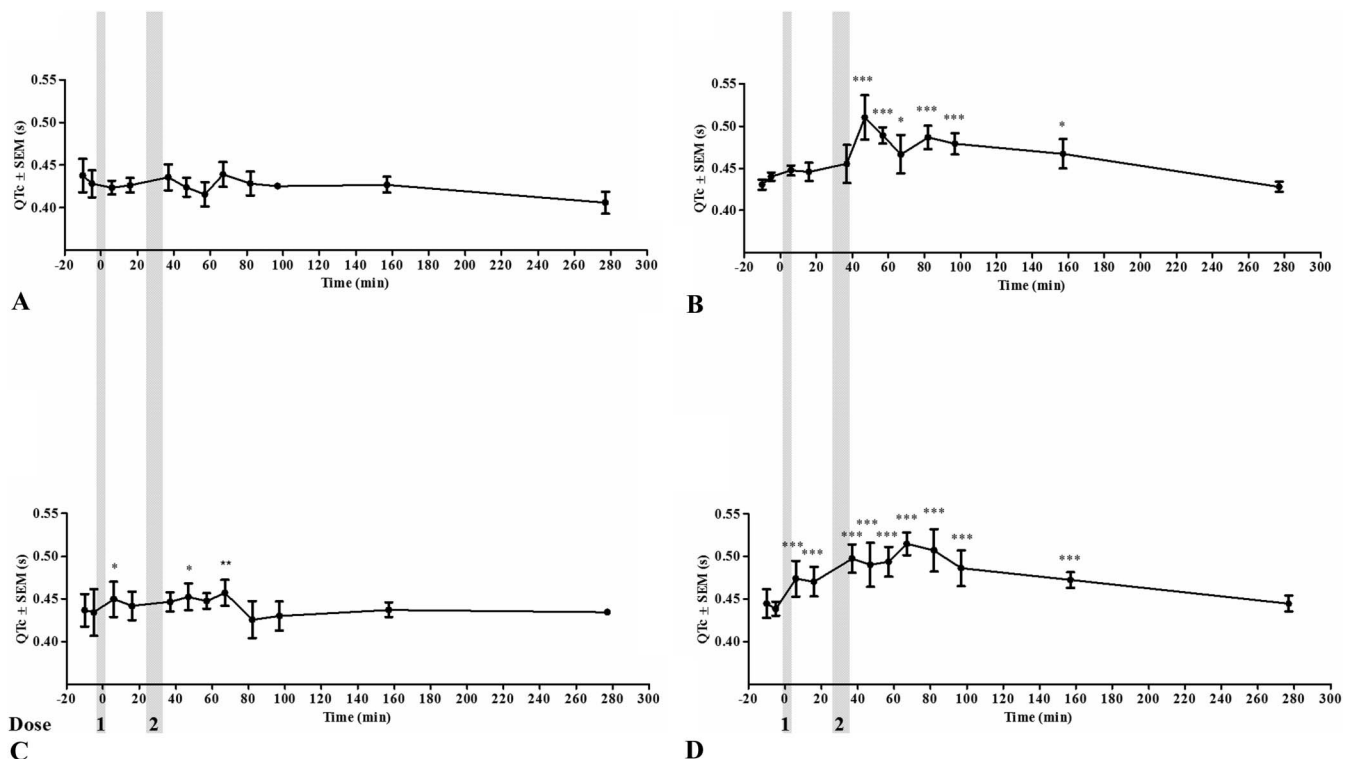
### ECG Analysis

ECGs were obtained throughout each procedure and for 24 hours after drug infusion using a Holter unit (Televet).<sup>15</sup> QT intervals and QRS durations were manually analyzed on lead II. Most measurements were made at

ventricular rates of 30–50 bpm (resting heart rate for horses is 28–40 bpm); however, the rates have been up to a maximum of 105 bpm. The QT measurements were corrected using a piecewise linear regression model specifically developed for horses. This regression model shows the lowest prediction errors at almost all heart rates when compared with other models.<sup>19</sup> Because of technical problems, ECGs were not available for analysis for 2 dofetilide and 2 ranolazine procedures.

### Data Analysis

All data are presented as mean ± standard error of measurement. Analyses were performed using GraphPad Prism 5 software (GraphPad Software, San Diego, CA) or R 3.3.1 software (The R Foundation for Statistical Computing, Vienna, Austria), with  $P \leq 0.05$  considered significant. Changes in QRS duration and corrected QT intervals (QTc) in the animals of the safety study were analyzed by 1-way repeated analysis of variance followed by a Dunnett’s multiple comparisons test using a time point of 5 minutes before drug injection, T(-5), as a reference. The QRS duration and QTc from the electrophysiology study were analyzed using a linear mixed model, and aERP data were analyzed by 2-way repeated-measures analysis of variance, followed by the Bonferroni post-test for pairwise comparisons. Changes in excitation threshold were analyzed using Wilcoxon signed-rank tests. AF vulnerability and AF duration were analyzed by Mann–Whitney  $U$  tests. Time to cardioversion was



**FIGURE 3.** Corrected QT intervals (QTc) during drug treatment. QTc during the 4 safety procedures: saline/control (A), dofetilide (B), ranolazine (C), and a combination of dofetilide and ranolazine (D). The gray areas represent dose administrations. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

analyzed by 1-way repeated analysis of variance, followed by Dunnett's multiple comparisons test.

## RESULTS

### Safety of Dofetilide and Ranolazine

The safety of dofetilide (8.0  $\mu\text{g}/\text{kg}$  BW) and ranolazine (2.4 mg/kg BW) for use in horses was first assessed by administering the compounds in 2 steps to healthy horses without AF, and monitoring them closely.

None of the horses in the study experienced adverse clinical reactions or an elevation of the cardiac biomarkers cardiac Troponin I and Creatinine Kinase fraction MB as a result of the drugs either alone or in combination (see **Material Figure 1, Supplemental Digital Content 1**, <http://links.lww.com/JCVP/A283>), and blood pressure remained unchanged after drug administration in the safety study (see **Material Figure 1, Supplemental Digital Content 1**, <http://links.lww.com/JCVP/A283>). The QRS duration and QTc in the safety study were prolonged by all treatments (Figs. 2 and 3). The T-wave morphology changed after dofetilide administration both alone and in combination in all 3 safety procedures (Figs. 4B, D). When the T wave was initially positive, it changed to a biphasic or negative deflection, and when it

was initially biphasic, it became negative. This phenomenon occurred despite a steady heart rate, and was not observed after administration of saline or ranolazine alone (Figs. 4A, C).

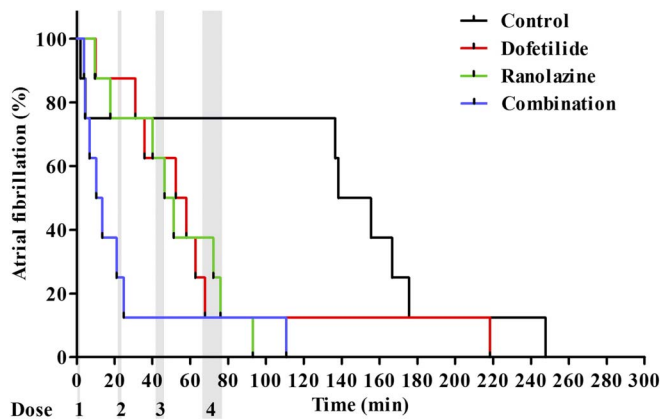
### Electrophysiology

The time to spontaneous cardioversion after the first drug injection was compared with the time to spontaneous cardioversion after saline injection in the time-matched control procedures (Fig. 5). The mean time to cardioversion in minutes was  $128.3 \pm 29.9$ ,  $67.0 \pm 22.7$ ,  $50.8 \pm 10.2$ , and  $24.5 \pm 12.6$  in the control, dofetilide, ranolazine, and combination procedure groups, respectively (see **Material Table S2, Supplemental Digital Content 1**, <http://links.lww.com/JCVP/A283>). Both ranolazine ( $P < 0.05$ ) and the combination treatment ( $P < 0.05$ ) significantly shortened the time to cardioversion. The combination of 0.889  $\mu\text{g}/\text{kg}$  dofetilide and 0.104 mg/kg ranolazine cardioverted 6 of 8 horses (dose 1 combination, Fig. 5), whereas the same doses of the drugs administered individually were only able to cardiovert 3 (dose 2 dofetilide, Fig. 5) and 2 horses (dose 1 ranolazine, Fig. 5), respectively. Infusion of 8.0  $\mu\text{g}/\text{kg}$  dofetilide did not result in cardioversion rates that were different from those in the control group.

The threshold of excitation, aERP, AF vulnerability, and AF duration were studied before treatment and after the



**FIGURE 4.** Changes in T-wave morphology following drug treatment. ECGs (lead II) from the same horse before and after administration of drugs: saline (A), dofetilide (B), ranolazine (C), and a combination of dofetilide and ranolazine (D). Note the changes in the T-wave morphology after dofetilide and combination treatment (B, D).

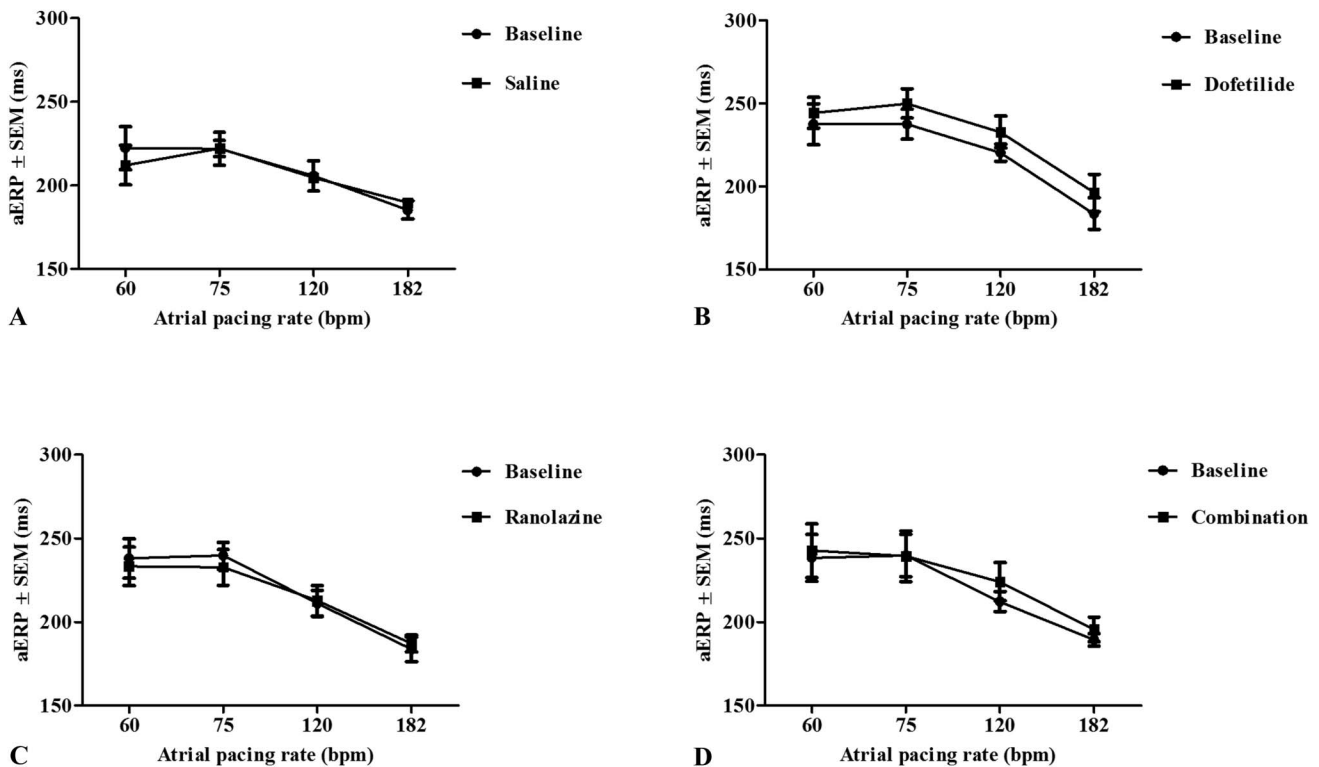


**FIGURE 5.** Time from start of drug administration to cardioversion. The percentage of horses remaining in AF as a function of time (in minutes). Time 0 is the start of the first drug administration. The gray areas represent the 4 dose administrations. In all groups n = 8.

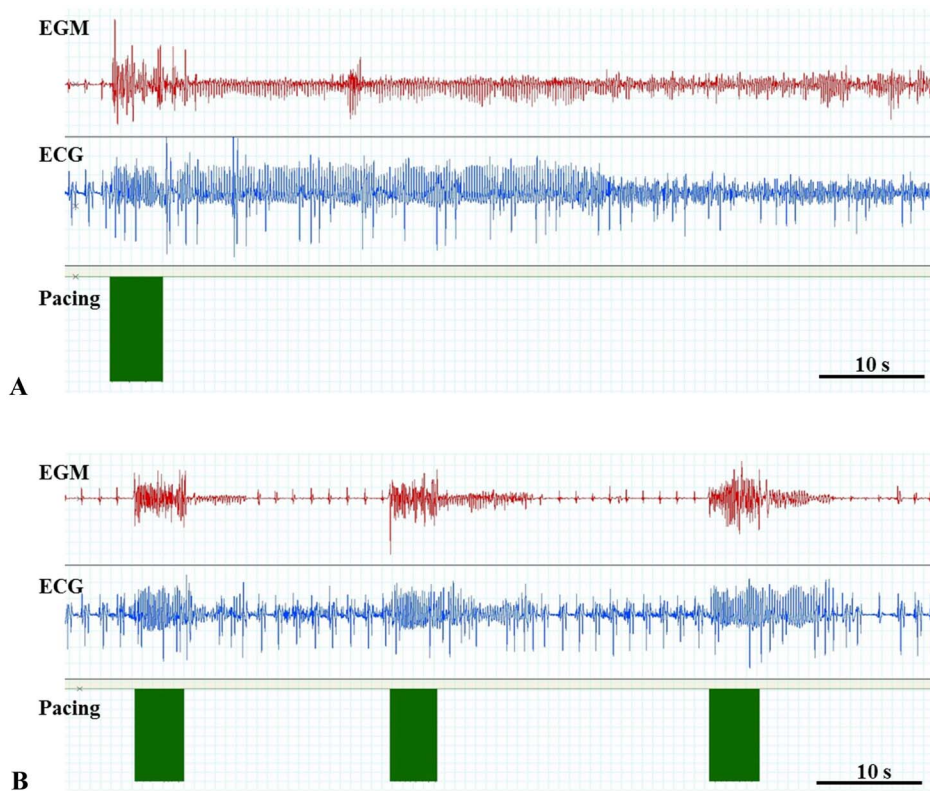
horses had received the full dose of each drug. It was not possible to perform the investigations after drug infusion in 6 saline, 1 dofetilide, and 1 combination procedure, because the horses were not in sinus rhythm. The threshold of excitation was in the range of 0.3–1.3 mA before drug treatment, and after drug treatment 0.3–1.1 mA ( $P > 0.05$  for all

procedures). In the control animals, aERP varied with pacing frequency from ~240 milliseconds at 60 bpm to ~180 milliseconds at 182 bpm (Fig. 6A). None of the treatments significantly prolonged aERP (Fig. 6). The combination treatment decreased AF vulnerability ( $P < 0.05$ ) and AF duration ( $P < 0.05$ ), whereas the drugs used individually had no effect on these parameters (Figs. 7 and 8).

With respect to ventricular electrophysiology, none of the treatments altered the QRS duration when compared with the control group, yet this was significantly prolonged in the ranolazine group when compared with the combination group ( $\Delta QRS_{ran} = 0.00049 \pm 0.001$  seconds,  $\Delta QRS_{combi} = -0.0020 \pm 0.001$  seconds,  $P < 0.05$ ; Fig. 9B). Dofetilide, ranolazine, and the combination treatment all significantly increased QTc when compared with the control group ( $\Delta QT_{c,dof} = 0.021 \pm 0.002$  seconds,  $\Delta QT_{c,ran} = 0.013 \pm 0.002$  seconds,  $\Delta QT_{c,combi} = 0.018 \pm 0.002$  seconds,  $P < 0.001$  for all 3 comparisons; Fig. 9C). No significant differences were found when comparing either drug used individually to the combination, but dofetilide prolonged the QTc more than ranolazine ( $P < 0.001$ ). The maximum QTc reached was  $0.43 \pm 0.01$  seconds in the control procedure,  $0.48 \pm 0.01$  seconds after dofetilide treatment,  $0.44 \pm 0.01$  seconds after ranolazine treatment, and  $0.45 \pm 0.01$  seconds after combination treatment. No ventricular arrhythmias were detected during or after any of the drug treatments.



**FIGURE 6.** Right aERP after drug treatment. The aERP was measured at different pacing rates before and after the infusion of drugs: saline (A), dofetilide (B), ranolazine (C), and the combination of dofetilide and ranolazine (D). A, Only 2 horses cardioverted within the time of the experiment, therefore, n = 8 at the baseline and n = 2 in the saline group. B, One horse did not cardiovert within the time of the experiment and 1 horse had unstable placement of the electrode: n = 6. C, n = 8. D, One horse did not cardiovert within the time of the experiment: n = 7.



**FIGURE 7.** Induction of AF. Electro-gram (EGM) from the right atrium, ECG, and pacing from the same horse before (A) and after (B) administration of the combination therapy with dofetilide and ranolazine. In both images tachypacing is performed leaving no visible gap between each stimuli. Note the short AF episodes (AF duration) after AF induction after combination therapy (B).

## DISCUSSION

This study is the first to examine the effects of combining dofetilide and ranolazine for the termination of AF. The combination protocol demonstrated antiarrhythmic potential by cardioverting horses in acutely induced AF and decreasing AF vulnerability and duration after drug administration.

### Combination Therapy in the Treatment of AF

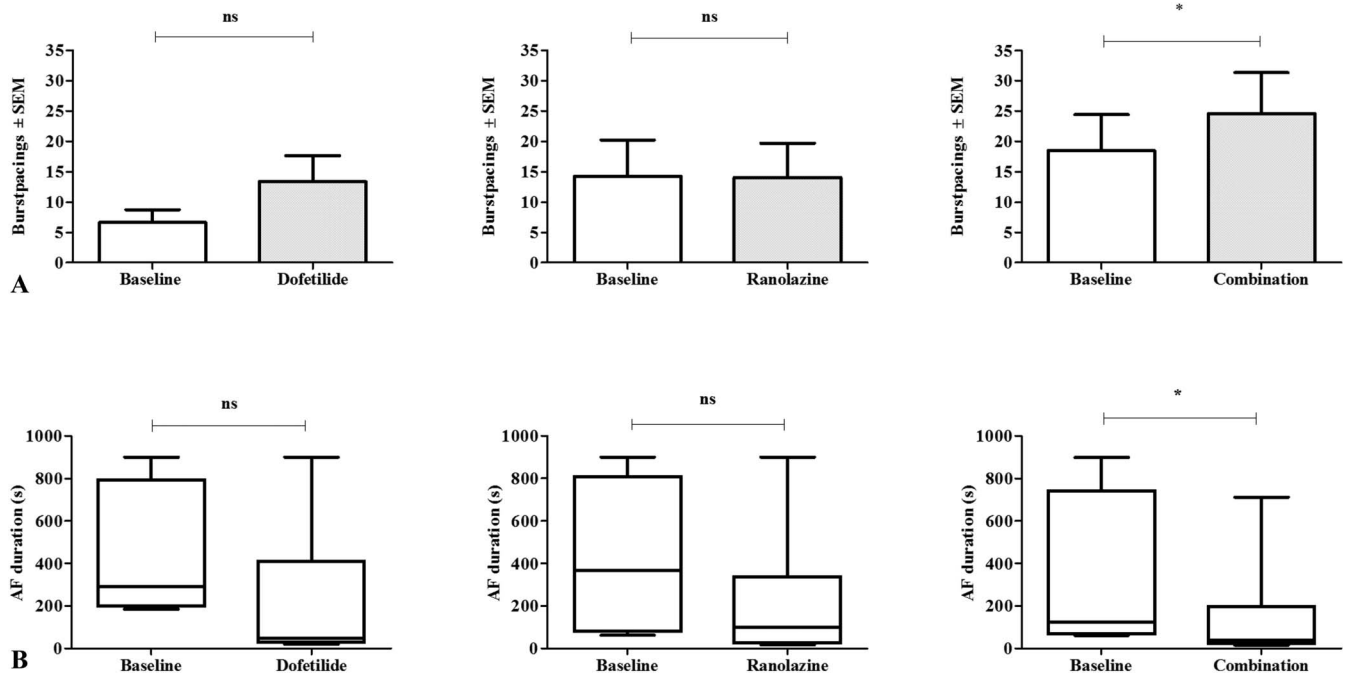
One case report has previously found the combination of dofetilide and ranolazine to be effective in maintaining sinus rhythm in a patient with a history of paroxysmal AF.<sup>20</sup> Several studies have combined ranolazine with amiodarone or dronedarone, finding the combination to be superior to either drug used individually.<sup>4,12,13,21,22</sup> Dofetilide has also been proposed for use in combination therapy for the termination of AF.<sup>23</sup> Previous studies combining antiarrhythmic compounds in the treatment of AF have shown synergistic effects.<sup>12,13,23,24</sup> In this study, we demonstrated an increased antiarrhythmic effect for the combination of dofetilide and ranolazine relative to monotherapy with either drug. We demonstrated that the combination of 0.889  $\mu\text{g}/\text{kg}$  dofetilide and 0.104 mg/kg ranolazine cardioverted 6 of 8 horses, whereas the individual use of these drugs at the same doses were only able to cardiovert 3 and 2 of the 8 horses, respectively. Furthermore, a decrease in AF vulnerability and duration only occurred after treatment with the combination of drugs. This protective mechanism against reinduction of AF corresponds well with previous findings of a combination strategy that was able to decrease the AF burden in patients with paroxysmal AF.<sup>4,20</sup>

### Ranolazine as Monotherapy in AF

Ranolazine has shown the ability to terminate AF and atrial flutter in a canine sterile pericarditis model,<sup>25</sup> and the drug was able to cardiovert induced AF to atrial flutter in anesthetized pigs.<sup>17</sup> In humans, a “pill-in-pocket” approach, using a single oral dose of ranolazine to cardiovert AF of acute onset (between 3 and 48 hours of AF) was successful in 72% of cases.<sup>5</sup> However, it is worth noting that no control group was included in this study, and spontaneous cardioversion is highly likely to have occurred in some of the patients.<sup>5</sup> Other studies have found ranolazine useful for maintenance of sinus rhythm,<sup>26,27</sup> whereas the AF burden was not decreased in the HARMONY trial after ranolazine administration alone.<sup>4</sup> In this study, 2.4 mg/kg of ranolazine produced a significant antiarrhythmic effect, which is in contrast to the individual use of dofetilide up to a dose of 8.0  $\mu\text{g}/\text{kg}$ . However, no effects on AF vulnerability or AF duration were found with the administration of either drug alone, indicating the lack of a protective effect on the initiation of new AF episodes.

### Atrial Electrophysiology and Underlying Mechanisms

Both dofetilide and ranolazine have been reported to cause an increase in aERP.<sup>3,17,18,21</sup> In this study, ranolazine resulted in aERP values similar to the baseline, and the 10–15-millisecond prolongation observed in aERP after dofetilide (Fig. 6B) did not reach statistical significance. As dofetilide alone did not display significant antiarrhythmic properties, it may be the case that the  $I_{K_r}$  antiarrhythmic



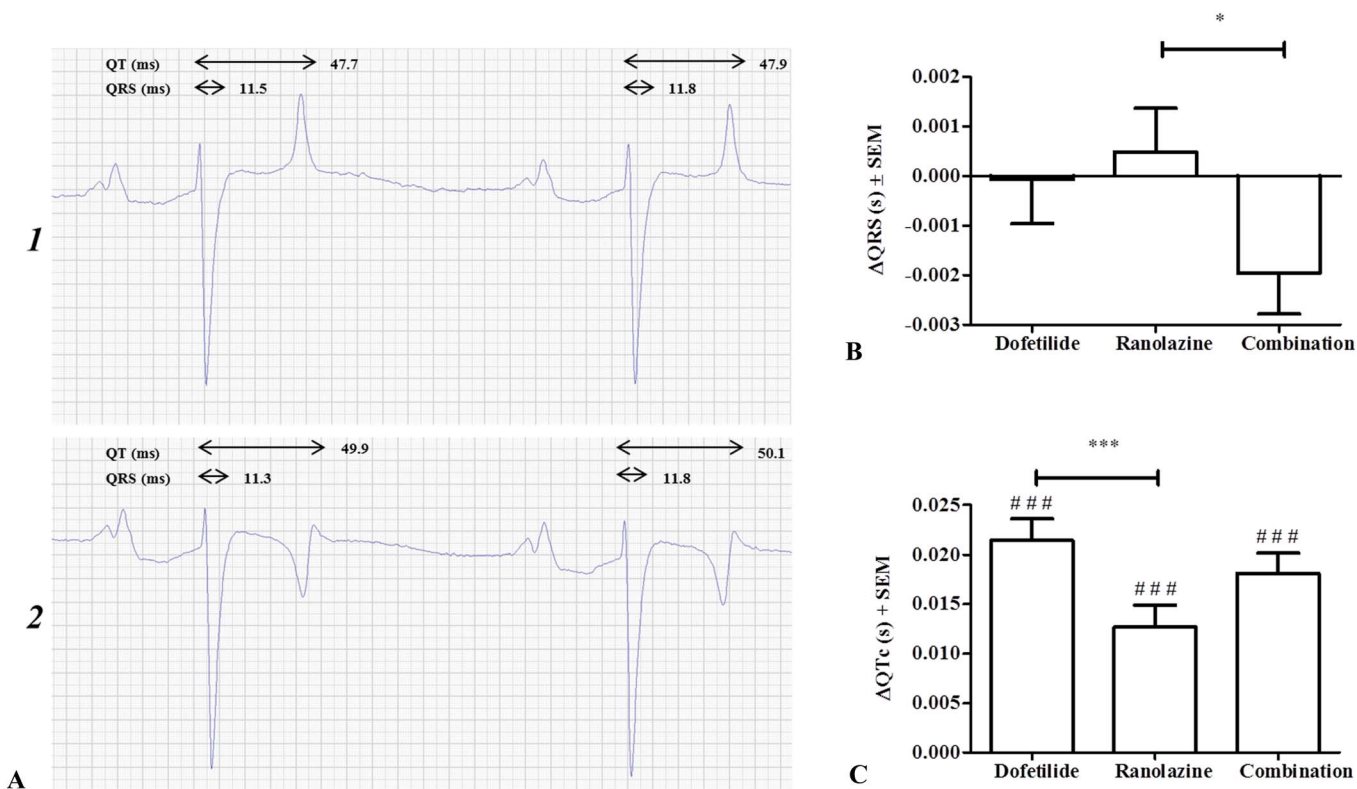
**FIGURE 8.** Atrial fibrillation (AF) vulnerability and mean AF duration after drug treatment. A, AF vulnerability presented as the mean number of burst-pacings required to induce an AF episode of  $\geq 15$  minutes. B, AF duration is presented in box plots and whiskers represent minimum to maximum values. In the dofetilide procedures  $n = 6$ , in the ranolazine procedures  $n = 7$  and in the combination procedures  $n = 7$ . \*  $P < 0.05$ ; ns = nonsignificant.

target is not expressed in a sufficient level in horse atria to lead to cardioversion. The chosen maximum dose was based on the recommendations for safe and efficacious dosing in humans,<sup>16</sup> which can be argued not to apply in horses. The expression of the gene encoding for the  $I_{K_r}$  ( $KCNH2$ ) in the equine atria has been shown,<sup>14,28</sup> but a higher expression of  $KCNH2$  was found in the right ventricle compared with the right atrial appendage,<sup>14</sup> in contrast to the uniform expression level in human atrial and ventricular myocardium.<sup>29</sup> The functional role of  $I_{K_r}$  has only been demonstrated in equine ventricular tissue,<sup>30</sup> and therefore, further studies are warranted. Despite an increased antiarrhythmic effect seen with the combination of drugs no changes in aERP were seen. It is unlikely that dofetilide acted in another way than  $I_{K_r}$  block and changes in repolarization. The possible explanations for the lack of effect on aERP are therefore; 1) dofetilide acted by blocking  $I_{K_r}$  and prolonging the action potential duration, but the prolongations were not sufficiently large to achieve statistical significance; 2) dofetilide acted through  $I_{K_r}$  block, but the effect was mediated by changes in action potential morphology that enhanced ranolazine-induced  $I_{Na}$  block but did not significantly prolong the action potential duration.<sup>31</sup> In a study by Aguilar et al,<sup>31</sup> it was shown that potassium channel block potentiates the AF-selective antiarrhythmic effects of optimized sodium-channel blockade. The mechanisms behind this synergism are rate dependent, and the effect is therefore largest in the atria during AF.

### Effect of Drugs on Ventricular Electrophysiology

It has been shown that dofetilide can cause TdP in humans.<sup>2</sup> No adverse cardiovascular or noncardiovascular reactions were encountered in the horses receiving ranolazine or dofetilide in this study. Both drugs displayed QTc-prolonging effects, in accordance with studies in other species.<sup>6,9,23</sup> Dofetilide prolonged the QTc more than ranolazine, presumably because dofetilide mainly blocks  $I_{K_r}$ , and this prolongs the entire repolarisation phase of the action potential. Conversely, ranolazine blocks  $I_{Na,late}$  with great potency in ventricular myocytes, thereby shortening the early repolarization.<sup>8,32</sup> None of the compounds prolonged the QRS interval, indicating that the ventricular effects were on the repolarization of the action potential. Human studies have shown similar QRS results after dofetilide administration, but a slight prolongation of the QRS duration after ranolazine administration.<sup>32</sup> It is worth noting that in the safety part of this study, some prolongation of the QRS interval was seen, but these results were based on only 3 horses (Fig. 2). Little is known about the T-wave morphology in horses, but it was evident that dofetilide changed the morphology in this study (Figs. 4B, D). Infusion of ranolazine also seems to change the morphology, as the T wave appears to have smaller amplitude (Fig. 4C). An  $I_{K_r}$  block induced by dofetilide or ranolazine is known to affect T-wave morphology in humans by causing concentration-dependent T-wave flatness, asymmetry, and notching.<sup>33</sup> The changes in T-wave morphology observed





**FIGURE 9.** Ventricular effects of drug treatments. A, ECG from the same horse before (1) and after (2) dofetilide administration with illustration of QRS duration and QT interval. Heart rate is 36 bpm in both ECGs. B, QRS duration and (C) corrected QT intervals (QTc) after drug treatment presented as the mean prolongation ± SEM compared with the control group receiving saline. #Represents significance levels of the comparison between the different drug treatments and the control group. \**P* < 0.05; \*\*\**P* < 0.001; ###*P* < 0.001.

in this study may be unique to horses and should be studied further to determine causes and consequences.

A recent epidemiological study found an increased risk of TdP related to the use of ranolazine in a population of patients with prolonged QTc.<sup>7</sup> By contrast, previous studies have reported that ranolazine reduces ventricular arrhythmias by suppressing early afterdepolarizations, despite the QTc-prolonging effect.<sup>6,9</sup> In a canine model, TdP was induced using dofetilide, but when ranolazine was added, the number of TdP episodes was significantly decreased.<sup>6</sup> No ventricular arrhythmogenic events were noted in this study, despite the observed QTc prolongation.

**Study Limitations**

The size of the study population poses an important limitation, which became apparent when 2 horses cardioverted very early in the control procedures. The decision to use 15 minutes of AF as the threshold for the start of treatment was based on previous studies,<sup>15,34</sup> yet it could be argued that this was too short a time in this study.

Knowledge of the pharmacodynamics of dofetilide and ranolazine in horses is currently lacking. More information on this would be valuable to compare the findings from this study to those in other species. In humans, the elimination half-life of dofetilide is around 10 hours,<sup>35</sup> and for ranolazine,

is around 1.5 hours.<sup>36</sup> As a consequence, the dosing regimen of this study (with 20 minutes in between treatments) is not considered to be problematic in terms of comparisons between dofetilide used alone and in combination. However, ranolazine plasma concentrations may not have reached the levels that could be expected if the total dose was given at once.

The level of electrophysiological analysis is too limited to define the exact mechanisms of interaction between dofetilide and ranolazine. The mechanisms suggested in the study therefore remain speculative. Furthermore, the mechanism of AF in this model is unknown and extrapolation of results should be performed with caution.

**CONCLUSIONS**

The combination of dofetilide and ranolazine showed clear antiarrhythmic effects—terminating acutely induced AF in horses, protecting from the reinduction of AF and shortening AF episodes induced after treatment. Ranolazine as a monotherapy was also found to terminate AF, but it did not display other antiarrhythmic effects. The use of the drugs individually and in combination prolonged the QTc, which may indicate the possibility of proarrhythmic events in the ventricles, although no such events were encountered.

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