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Effects of dofetilide and ranolazine on atrial fibrillatory rate in a horse model of acutely induced atrial fibrillation

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Abstract

Introduction: The atrial fibrillatory rate is a potential biomarker in the study of antiarrhythmic drug effects on atrial fibrillation (AF). The purpose of this study was to evaluate whether dose-dependent changes in the atrial fibrillatory rate can be monitored on surface electrocardiography (ECG) following treatment with dofetilide, ranolazine, and a combination of the two in an acute model of AF in horses.

Methods and Results: Eight horses were subjected to pacing-induced AF on 4 separate days. Saline (control), dofetilide, ranolazine, or a combination of dofetilide and ranolazine was administered in four incremental doses. Atrial fibrillatory activity was extracted from surface ECGs using spatiotemporal QRST cancellation. The mean atrial fibrillatory rate before drug infusion was 297 ± 27 fpm. Dofetilide reduced the atrial fibrillatory rate following the infusion of low doses (0.89 μg/kg, $P < 0.05$) and within 5 minutes preceding cardioversion ($P < 0.05$). Cardioversion with ranolazine was preceded by a reduction in the atrial fibrillatory rate in the last minute ($P < 0.05$). The combination of drugs reduced the atrial fibrillatory rate in a similar manner to dofetilide used alone. A trend toward a lower atrial fibrillatory rate before drug infusion was found among horses cardioverting on low doses of the drugs.

Conclusion: The atrial fibrillatory rate derived from surface ECGs showed a difference in the mode of action on AF between dofetilide and ranolazine. Dofetilide reduced the atrial fibrillatory rate, whereas ranolazine displayed a cardioverting mechanism that was distinct from a slowing of the fibrillatory process.

KEYWORDS
atrial fibrillation, atrial fibrillatory rate, combination therapy, dofetilide, ranolazine

1 | INTRODUCTION

Pharmacological treatment is important in the control of atrial fibrillation (AF) and restoration of sinus rhythm. However, the antiarrhythmic drugs currently available display varying efficacy and most, if not all, can cause severe adverse reactions.[1] This has created an emerging interest in combining two antiarrhythmic drugs to create a synergistic effect, thereby increasing efficacy while potentially avoiding toxicity through the use of lower doses of each drug.[2] Our group, recently studied the antiarrhythmic effects of combining dofetilide and ranolazine in a model of induced AF in horses and found that the combination increased the efficacy of the drugs.[3] Dofetilide is a Class III antiarrhythmic drug that selectively blocks the rapidly activating delayed rectifier potassium current (I_{Kr}) and is known to increase the QT interval and the risk of ventricular arrhythmias.[1] Ranolazine blocks the late sodium current ($I_{Na,late}$) and in higher doses also I_{Kr}. Furthermore, ranolazine potently blocks the peak sodium current ($I_{Na,peak}$) in the atria.[4,5]

In recent years, substantial attention has been paid to monitoring the atrial activity on surface electrocardiography (ECG) during AF. Measuring the ECG-derived atrial fibrillatory rate (AFR) is a noninvasive method and has been used to study the electrical remodeling that occurs during AF.[6–8] Furthermore, it has been proposed that AFR can be used to assess antiarrhythmic drug effects[9–12] and may predict the likelihood of drug-induced cardioversion.[7] The alterations in atrial activity evident on the surface ECG following administration of a number of antiarrhythmic drugs (including dofetilide and ranolazine) have been studied,[9,10,13–15] yet no such studies on the use of combination therapies are available.

The horse has been suggested as a model of human AF, as, like humans, horses develop both lone AF and AF as a consequence of underlying structural heart disease.[16] Experimental induction of AF is easily performed in horses and has been used in several pharmacological studies.[3,17–19] Furthermore, it has recently been shown that AFR dynamics in horses with experimentally induced AF resemble AFR dynamics in humans with paroxysmal AF and that flecainide administration has a very similar effect on AFR in the two species.[20]

In the present study, we aimed to investigate the effects of dofetilide and ranolazine, both alone and in combination, on AFR in a model of acutely induced AF in horses.

2 | MATERIALS AND METHODS

ECGs obtained from eight standardbred horses (body weight 492 ± 32 kg, age 8.9 ± 3.4 years; five mares and three geldings) were included in the study. Data were collected from previously published experiments and the design of the study has, therefore, been described.[3] Furthermore, five ECGs from the control experiments were previously used for studying AFR dynamics in horses.[20] In brief, all horses were subjected to four procedures with a 1-week washout period in between. All procedures were performed on standing nonsedated horses restrained in a stock. During each procedure, the horses were instrumented with two multipolar steerable nonfixative electrodes (Inquiry Steerable Diagnostic Catheter, 6 Fr/110 cm; St. Jude Medical, St. Paul, MN) in the right atrium; one for pacing and one for obtaining an atrial electrogram, and AF was induced by burst-pacing with 50 Hz for 4 to 6 seconds. Once an episode of AF with a duration of over 15 minutes was observed, drug treatment was initiated with either saline (NaCl 9 mg/mL; B. Braun, Melsungen, Germany), dofetilide (Catalog No. 3757; Tocris Bioscience, R&D Systems, Abingdon, UK), ranolazine (Catalog No. 3118; Tocris Bioscience, R&D Systems), or a combination of dofetilide and ranolazine. All drugs were administered over four stages with 20 minutes and a threefold increase in the total dose between each stage being observed. The total dose of dofetilide was 8.00 μg/kg and the total dose of ranolazine was 2.40 mg/kg. Saline was administered at a total dose of 0.20 mL/kg as this most closely resembled the volume of the drugs used. In the combination procedure, ranolazine was given at an identical dose as when used alone and 0.89 μg/kg of dofetilide was given with the first dose of ranolazine. All solutions were administered intravenously at a rate of 0.0125 mL/kg/min. All horses received all four doses regardless of the time of cardioversion. Horses that cardioverted within 30 minutes of the last dose being administered were classified as responders as opposed to the nonresponders that did not cardiovert within this time period.

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.

2.1 | ECG recordings and analysis

A surface ECG was obtained throughout each procedure using a Holter unit (Televet, Maarslev, Denmark).[17] Identical recordings were obtained simultaneously using LabChart 7 software (ADInstruments, Oxford, UK). The digital ECG from each AF episode from induction to cardioversion was exported and processed using AFR Tracker software (CardioLund Research AB, Lund, Sweden) to obtain the AFR and exponential decay (ED), which is a measure of organization of the rhythm.[10] The processing method using spatiotemporal QRST cancellation has been described elsewhere[6,8] and was previously used to analyze equine ECGs.[20] In this study, the AFR represents the average rate for 1-minute intervals.

2.2 | Data analysis

All data are presented as mean ± SD. All analyses were performed using GraphPad Prism 5 software (GraphPad Software, San Diego, CA), with $P \leq 0.05$ considered to be significant. All mean AFR and ED values are a mean of five consecutive minutes unless stated otherwise. The mean AFR and ED before treatment were compared for the four different

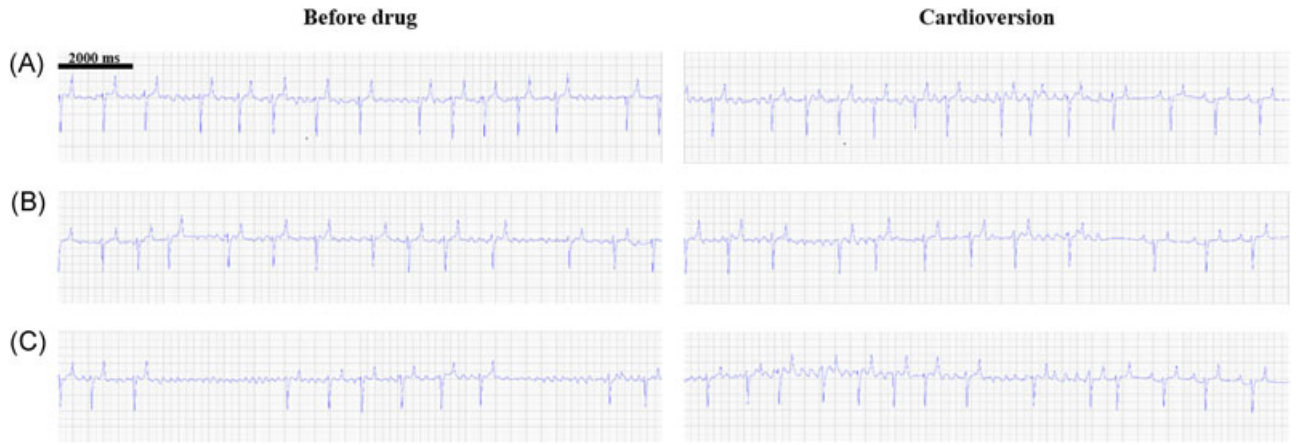

FIGURE 1 Electrocardiographic examples from drug procedures. ECG recordings from the same horse before drug infusion and during cardioversion in all three drug procedures; dofetilide (A), ranolazine (B), and the combination of dofetilide and ranolazine (C). ECG, Electrocardiography

procedures using a one-way repeated-measures analysis of variance followed by a Bonferroni posttest for pairwise comparisons. A moving median of five consecutive minutes was used for graphical illustration of the AFR for each individual horse during the different procedures. The mean AFR was compared before and after administration of each dose using paired *t* tests. Similarly, the mean ED was compared before and after administration of the drug doses. Using unpaired *t* tests, the mean AFR before drug infusion was compared for responders and non-responders in the control procedures as well as for animals cardioverting before and following the third dose in the dofetilide and ranolazine procedures.

3 | RESULTS

As previously shown,[3] two out of eight horses cardioverted during the control procedures, whereas the remaining six horses remained in AF for more than 1 hour following the last saline administration. Seven of the eight horses restored sinus rhythm during dofetilide treatment, eight during ranolazine treatment, and seven during the administration of the two drugs combined. An example of ECG recordings from each of the three drug procedures is given in Figure 1.

The mean AFR before drug treatment (including all procedures performed on all horses) was 297 ± 27 fpm and no differences were found in the mean AFR before drug treatment among the four procedures (control, dofetilide, ranolazine, and the combination of dofetilide and ranolazine; $P = 0.29$). Development of the AFR during the different drug treatments is illustrated for each horse in Figures 2 to 5. The mean ED before drug treatment was not different among the four procedures ($P = 0.66$).

3.1 | Control

In the graphical illustration of AFR during the control procedures, it is seen that the AFR of the nonresponders remained relatively stable throughout the procedures (Figure 2). This is demonstrated by comparisons in Table 1, finding no differences in mean AFR before and after each of the four saline infusions. For the two horses cardioverting after the first saline administration (ID1 and ID6), AFR started to decrease in 5 and 11 minutes, respectively, before the administration was initiated (Figure 2).

The mean ED before drug infusion was 1.34 and there were no differences found when comparing ED before and after the four saline doses.

3.2 | Dofetilide

Administration of a very low dose of dofetilide (dose 1, 0.30 μg/kg) was not accompanied by any consistent decrease in AFR during or following drug infusion, as visualized in Figure 3 and substantiated in Table 1. The horse that cardioverted after dose 1 (ID3) presented with a decreasing AFR trend that started 2 minutes before drug infusion. A decrease in mean AFR was evident after dose 2 of dofetilide (total dose received 0.89 μg/kg; Table 1). However, this decrease was not apparent in all horses (ID8). In ID2 and ID7 the decrease started 7 and 3 minutes before drug administration, respectively (Figure 3). A decrease in AFR initiated during drug infusion was consistently observed in five horses (ID1/2/4/5/8) that received dose 3 (total dose received 2.67 μg/kg) while in AF, with AFR stabilizing at a lower level (ID2/4/5/8) or returning to the preinfusion level (ID1) at the end of dose 3 infusion (Figure 3 and Table 1). A further decrease in AFR was seen in the nonresponder (ID8) following dose 4 but this was not followed by a restored sinus rhythm (total dose received was 8.00 μg/kg; Figure 3).

A comparison of the mean AFR before the first drug infusion and the mean AFR in the 5 minutes leading up to cardioversion for all responders revealed a significant decrease in AFR. This was also the case for the last minute before cardioversion (Table 2). Studying the decrease in AFR in the individual animals it was found that horses with an AFR less than 300 fpm before the first drug infusion showed

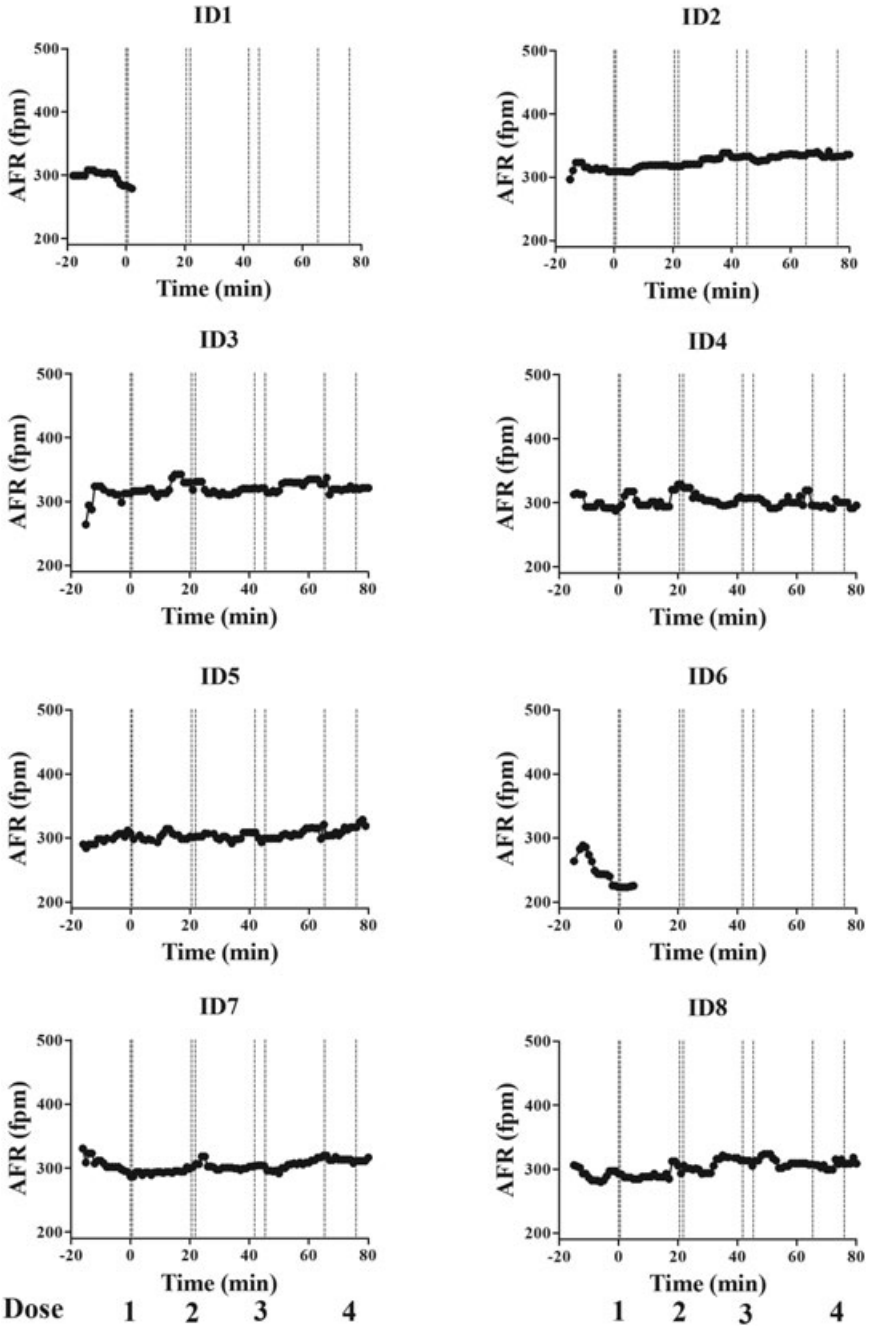

FIGURE 2 Atrial fibrillatory rate (AFR) during control procedures. Each graph illustrates the moving-median AFR in individual horses during the control procedures with time on the *x*-axis and fibrillations per minute (fpm) on the *y*-axis. The dotted lines mark the start and end of the drug infusions

a decrease in AFR leading up to cardioversion of between 14% and 24%. In horses with an AFR of above 300 fpm before drug infusion, the reduction was between −8% and 12%.

The mean ED before drug infusion was 1.40. No differences were found between ED before and after the four doses and neither when comparing ED before dofetilide infusion to the mean ED in the 5 minutes or last minute leading up to cardioversion.

3.3 | Ranolazine

There was no distinct decrease in AFR following drug infusions in the ranolazine procedures for the majority of the horses (Figure 4). This finding corresponds to the lack of change in mean AFR before and after the first and second drug infusions presented in Table 1. In ID2, ID3, and ID8, however, there was a decrease in AFR related to dose 3 (total dose received 0.94 mg/kg), and to dose 4 (total dose received 2.40 mg/kg) in ID2 and ID8 (Figure 4 and Table 1).

When comparing the mean AFR before the first drug infusion to the mean AFR in the 5 minutes leading up to cardioversion, no decrease was found. Conversely, AFR was significantly reduced in the last minute leading up to cardioversion (Table 2). Comparing the AFR before drug infusion to the AFR before cardioversion did not reveal any differences in reduction of the AFR between horses with an initial AFR above or below 300 fpm.

The mean ED before drug infusion was 1.39. No differences were found between ED before and after the four doses or between ED

TABLE 1 Comparisons of atrial fibrillatory rates before and after drug infusion

	Dose 1			Dose 2			Dose 3			Dose 4		
	AFR before	AFR after	*P*	AFR before	AFR after	*P*	AFR before	AFR after	*P*	AFR before	AFR after	*P*
Control	300 ± 9[a]	299 ± 11[a]	0.82	309 ± 12[a]	313 ± 9[a]	0.37	314 ± 13[a]	311 ± 10[a]	0.59	318 ± 13[a]	317 ± 13[a]	0.76
Dofetilide	301 ± 39[b]	301 ± 42[b]	0.90	310 ± 42[c]	296 ± 37[c]	**0.015**	323 ± 49[d]	297 ± 49[d]	**0.0008**	281[#]	264[#]	…
Ranolazine	304 ± 28[b]	301 ± 29[b]	0.50	304 ± 24[a]	300 ± 21[a]	0.12	331 ± 12[e]	312 ± 11[e]	**0.013**	339[#]	320[#]	…
Combination	290 ± 19[a]	278 ± 21[a]	**0.041**	303[#]	301[#]	…	315[#]	304[#]	…	327[#]	302[#]	

Abbreviation: AFR = atrial fibrillatory rate.
The AFR used for the comparisons are averages over 5 minutes and given in fibrillations per minute ± SD.
[a]$n = 6$.
[b]$n = 8$.
[c]$n = 7$.
[d]$n = 5$.
[e]$n = 4$.
[#]$n = 1$.
Bold numbers indicate statistical significance ($P < 0.05$).

before ranolazine infusion and the mean ED in the 5 minutes or last minute leading up to cardioversion.

3.4 | Combination therapy

A more (ID1/3/4) or less (ID2/6) pronounced decrease in AFR following the first combination dose of dofetilide (0.89 μg/kg) and ranolazine (0.104 mg/kg) was seen in five out of eight horses and in another animal (ID5), this decrease was initiated 3 minutes before drug administration (Figure 5 and Table 1). These findings were not consistent in ID7 and ID8 (Figure 5). In the horse that received all four drug doses while in AF (ID2), a slight decrease in AFR was seen in relation to all dose administrations.

The mean AFR of the 5 minutes leading up to cardioversion for the responders was lower when compared with the mean AFR before the first combination infusion. Similarly, AFR was lower in the last minute before cardioversion (Table 2). Comparing the reduction in AFR between horses displaying an AFR below and above 300 fpm before drug infusion did not reveal any differences.

The mean ED before the first drug infusion was 1.36. No differences were found between ED before and after the four doses of combination therapy. However, the ED in the 5 minutes and in the last minute leading up to cardioversion was significantly different from the ED before the first drug infusion ($P = 0.019$ and 0.039, respectively).

3.5 | AFR as a predictive measure

No significant differences were found between responders and nonresponders in the control procedures when comparing the mean AFR before drug infusion. The responders had an AFR of 265 ± 43 fpm, whereas AFR for the nonresponders was 300 ± 9 fpm ($P = 0.45$). For the dofetilide procedures, AFR was 298 ± 41 and 325 fpm for the responders and nonresponder, respectively. As ranolazine terminated AF in all animals, a comparison could not be made with this treatment. In the combination procedures, AFR was 288 ± 17 and 308 fpm for the responders and nonresponder, respectively.

In the dofetilide and ranolazine procedures, the mean AFR before drug infusion was compared for animals cardioverting before and following dose 3 to test AFR as a predictor of the dose required for cardioversion. In the dofetilide procedures, animals that cardioverted before ($n = 3$) and following ($n = 5$) dose 3 had a mean AFR of 278 ± 30 and 315 ± 39 fpm, respectively ($P = 0.21$). In the ranolazine procedures, the mean AFR was 283 ± 34 fpm for animals that cardioverted before dose 3 ($n = 3$) and 316 ± 16 fpm for those that cardioverted after dose 3 ($n = 5$; $P = 0.10$).

4 | DISCUSSION

This study is the first to assess the effect of ranolazine, both on its own and in combination with dofetilide, on the AFR retrieved from surface ECG preceding cardioversion. A clear slowing of the atrial fibrillatory process before cardioversion was observed with dofetilide alone and in

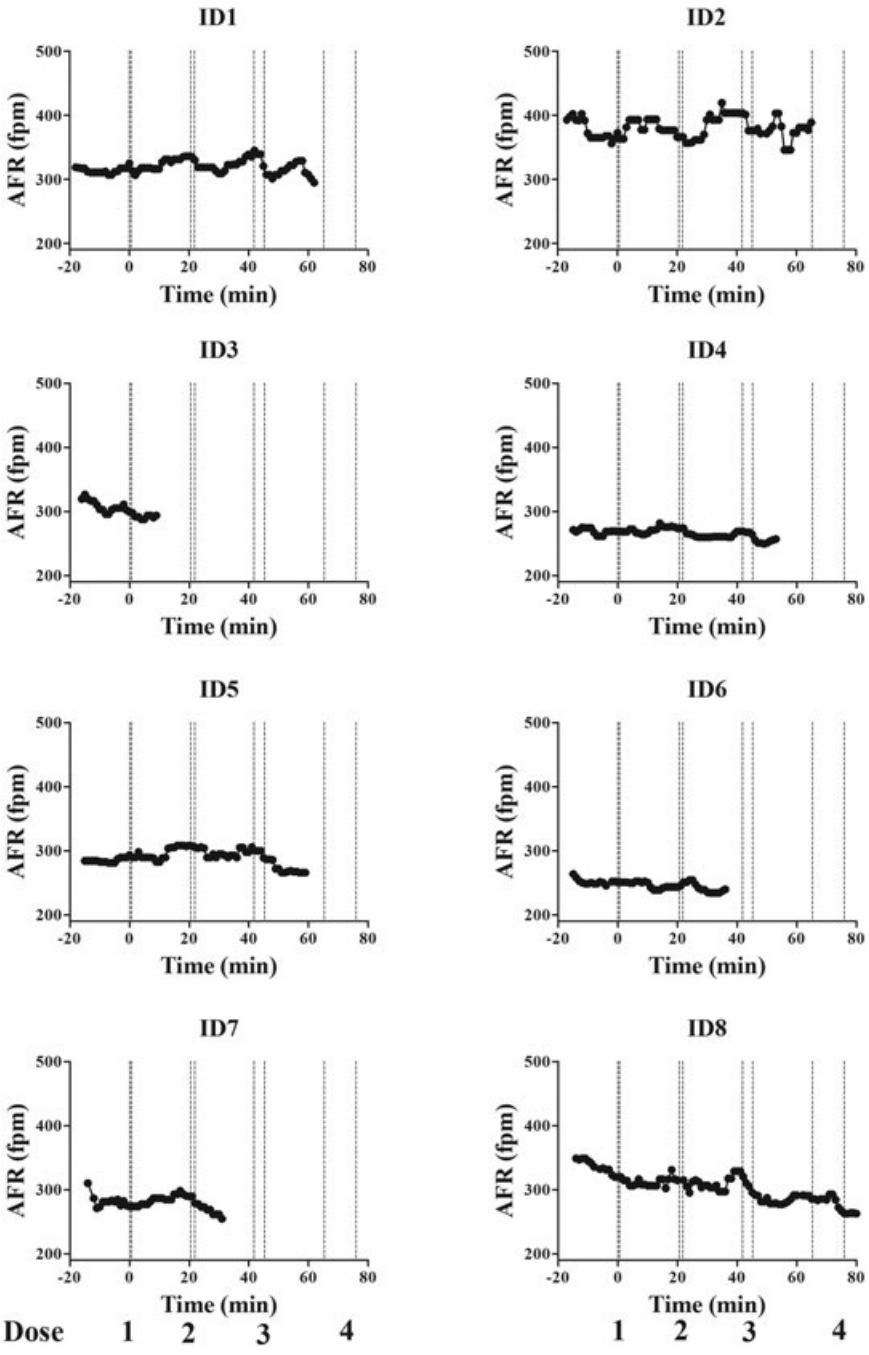

FIGURE 3 Atrial fibrillatory rate (AFR) during dofetilide procedures. Each graph illustrates the moving-median AFR in individual horses during the dofetilide procedures with time on the *x*-axis and fibrillations per minute (fpm) on the *y*-axis. The dotted lines mark the start and end of the drug infusions

TABLE 2 Atrial fibrillatory rate before drug infusion and at cardioversion in responders

	AFR before	AFR last 5 minute	AFR last 1 minute	Before/last 5 minutes (*P*)	Before/last 1 minute (*P*)
Dofetilide (*n* = 7)	298 ± 41	286 ± 50	276 ± 45	0.02*	0.01*
Ranolazine (*n* = 8)	304 ± 28	299 ± 29	276 ± 37	0.37	0.02*
Combination (*n* = 7)	288 ± 17	269 ± 21	254 ± 20	0.04*	0.01*

Abbreviations: AFR, atrial fibrillatory rate; AFR before, average of AFR 5 minutes before drug infusion; AFR last 1 minute: the last AFR value before cardioversion; AFR last 5 minutes: average of AFR 5 minutes before cardioversion.
Values are given in fibrillations per minute ± SD.
*Statistical significance ($P < 0.05$).

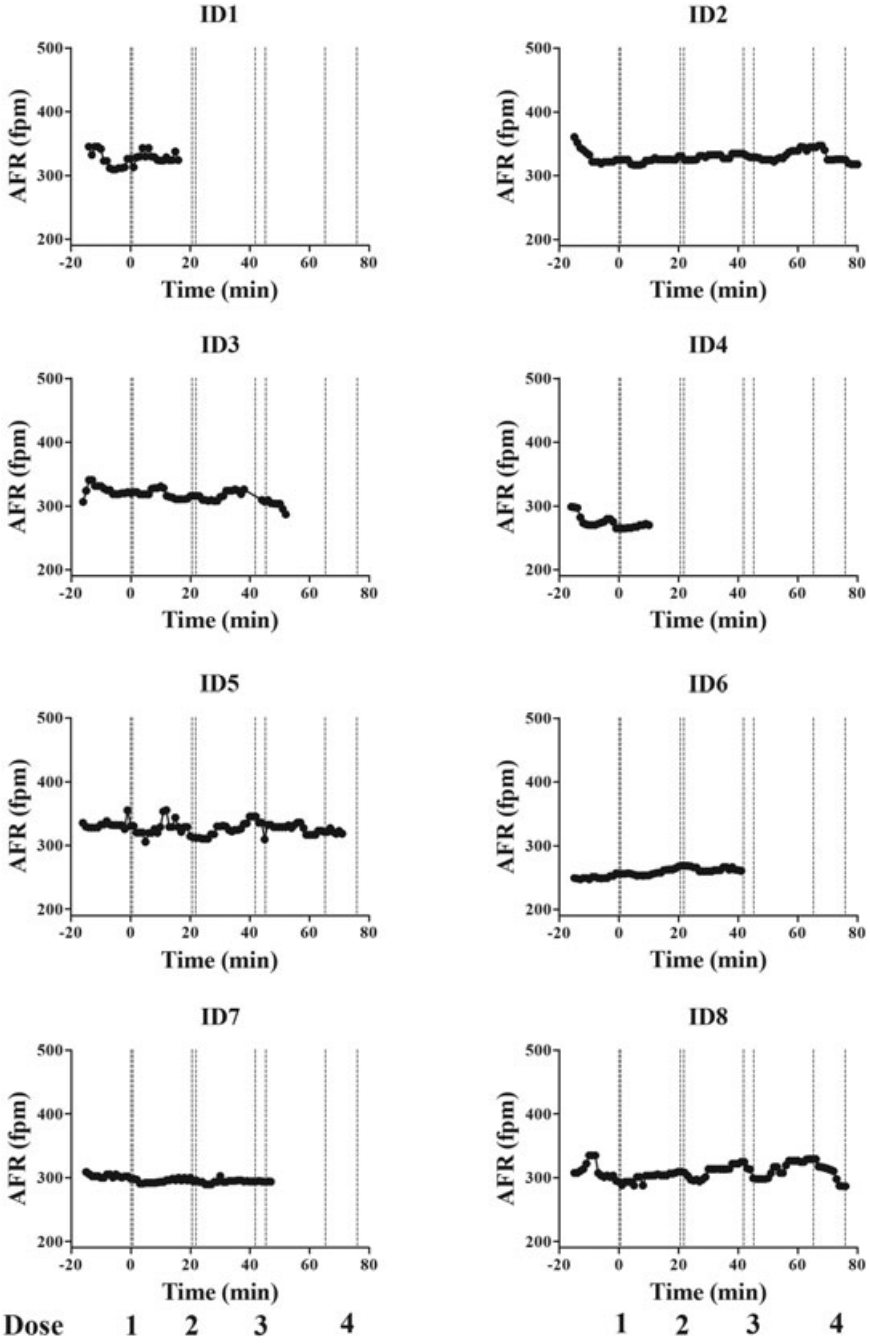

FIGURE 4 Atrial fibrillatory rate (AFR) during ranolazine procedures. Each graph illustrates the moving-median AFR in individual horses during the ranolazine procedures with time on the *x*-axis and fibrillations per minute (fpm) on the *y*-axis. The dotted lines mark the start and end of the drug infusions

combination with ranolazine, yet this mechanism was not consistently found with the use of ranolazine alone.

4.1 | Use of AFR to monitor antiarrhythmic drug effects

Previous studies looking into the effects of dofetilide on the atrial fibrillatory process have found a significant decrease in AFR after drug administration.[13,21] These findings are consistent with the present study, where dofetilide administration led to a dose-dependent AFR decrease: no measurable effect following dose 1, a 4.5% reduction in AFR following dose 2 and an 8.0% reduction in AFR following dose 3 (Table 1). Like other potassium channel blockers, dofetilide is known to increase atrial refractoriness.[1] However, no changes in the atrial effective refractory period were found following dofetilide administration in horses,[3] which could call into question the functional role of I_{Kr} in equine atria. In contrast, AFR modification following dofetilide administration indicates that I_{Kr} does indeed play a role in the equine atria, as blocking the current decreases AFR. Furthermore, it appears that dofetilide could display negative use-dependence in horses as reported in other species[22] as we saw a larger reduction in AFR in horses with a slower initial AFR. This

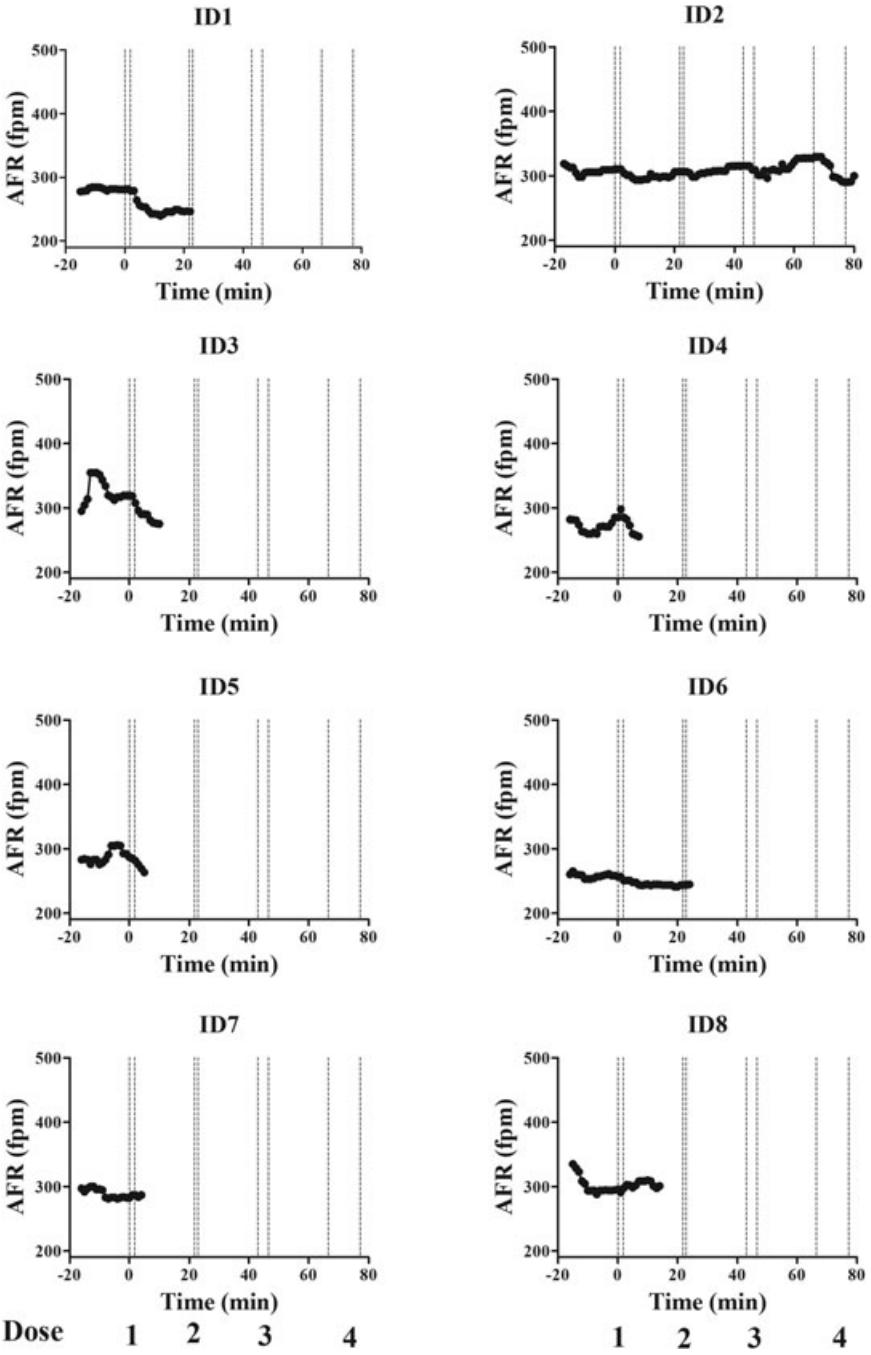

FIGURE 5 Atrial fibrillatory rate (AFR) during combination procedures. Each graph illustrates the moving-median AFR in individual horses during the combination procedures, with time on the *x*-axis and fibrillations per minute (fpm) on the *y*-axis. The dotted lines mark the start and end of the drug infusions

relationship is well described for I_{Kr} blockers, in general but has not previously been studied in horse atria.

In contrast with dofetilide, ranolazine infusion in low doses was associated with barely measurable effects on AFR in the majority of animals. Conversely, a decrease was seen following higher doses of ranolazine. In a study by Black-Maier et al[15] ranolazine was found to decrease the dominant atrial fibrillatory wave frequency in AF patients, which resembles our findings with the higher doses of ranolazine. When analyzing AFR during the time preceding cardioversion, a reduction was only seen during the last minute before cardioversion, while dofetilide administration was associated with a nearly immediate and more pronounced reduction in AFR. This indicates a difference between the two antiarrhythmic drugs in the effect on atrial electrophysiology and in the way they terminate AF. Ranolazine blocks $I_{Na,late}$ and I_{Kr} but also has a blocking effect on $I_{Na,peak}$ in the atria.[4,5] Dofetilide is reported to be a selective I_{Kr} channel blocker when applied acutely,[1] while chronic exposure may increase $I_{Na,late}$.[23] One can speculate that the reduction in AFR following high-dose ranolazine administration (which mimics AFR behavior induced by dofetilide) may reflect the increased I_{Kr} blockade. There is conflicting clinical experience on the effect of other combined ion channel blockers on AFR during AF. The combined potassium and sodium blocker AZD7009 has been found to significantly reduce AFR,[9] whereas bepridil,

which has sodium, potassium, and calcium channel blocking properties did not reduce AFR significantly.[24]

When dofetilide and ranolazine were combined, AFR was significantly reduced in 5 minutes leading to cardioversion, resembling the effect when dofetilide was used alone. This effect was expected, as dose 1 of dofetilide in the combination procedures (0.89 μg/kg) was identical to the dofetilide dose the horses had received following the second infusion in the dofetilide-only procedures, in which a clear trend of AFR reduction was seen in the majority of horses (Figure 3). Interestingly, the reduction in AFR did not show any relation to the initial AFR of the individual horses suggesting that the addition of ranolazine counterbalances the reverse use-dependence of dofetilide. Furthermore, the combination of drugs resulted in a decreased ED before cardioversion reflecting a greater organization of the AF during this time. This finding may be part of the explanation of the increased antiarrhythmic effects of the combination of dofetilide and ranolazine.

In a study by Wijffels et al[25] the antiarrhythmic effects of Classes I and III antiarrhythmic drugs were not explained by a prolongation of the atrial wave length (wave length = refractory period × conduction velocity[26]) but rather by a widening of the temporal excitable gap, which is the difference between the atrial fibrillatory cycle length and the refractory period. We have only studied the atrial refractoriness after cardioversion to sinus rhythm but did not find any significant prolongation.[3] An explanation for the antiarrhythmic effects could, therefore, be an increase in the temporal excitable gap because the atrial fibrillatory cycle length is increased (the AFR is decreased) to a smaller or larger degree by all the drugs in our study. However, we would need to measure the conduction velocity and possible alterations following drug infusions to be able to conclude whether the wave length and path length were altered. The difference between these two parameters reflects the spatial excitable gap, which was found to increase significantly with the use of D-sotalol (Class III) but not flecainide (Class I) in goats.[25]

4.2 | Prediction of drug doses for cardioversion

In a study by Bollmann et al[7] on the effects of Class III antiarrhythmic drug ibutilide, the baseline fibrillatory rate was lower in patients that cardioverted solely on antiarrhythmic therapy than in patients who failed to cardiovert on drugs and required electrical cardioversion. In contrast, Raygor et al[13] found no such difference in their study on dofetilide and in a study of another Class III antiarrhythmic compound vernakalant, no difference could be found between the baseline AFR of responders and nonresponders.[14] Similar findings were observed in a study of AZD7009.[9] The present study addressed the possible difference between horses requiring lower or higher doses of dofetilide or ranolazine for cardioversion by comparing the mean AFR before drug infusion for horses cardioverting on a low dose and for horses not cardioverting, or cardioverting on higher doses of the studied drugs. We could not confirm any significant difference in the mean AFR before drug infusion between these two groups but the AFR values were lower in animals exhibiting a restored sinus rhythm on the lower doses. However, due to the small sample size, a definitive conclusion on the prognostic value of AFR for predicting the drug effect cannot be drawn.

4.3 | Spontaneous cardioversion in the horse model

All horses in the present study cardioverted spontaneously, if not by drug intervention. Hesselkilde et al[20] found AFR in horses with induced AF to be comparable to AFR in humans with paroxysmal AF, suggesting similar AFR cut off values for spontaneous cardioversion around 350 fpm.[27] In addition, the horses with induced AF had lower AFR values compared to horses with spontaneous persistent AF.[20] The mean AFR before drug infusion in the present study was 297 ± 27 fpm, so spontaneous cardioversion was expected. A study of ten horses showed that acutely induced AF with a duration of over 15 minutes will terminate after more than 1 hour,[18] and another study of three horses found the time to cardioversion of AF episodes of more than 15 minutes to be 5.8 ± 3.2 hours.[17] In the present study, two horses cardioverted spontaneously (following saline administration) after 20 minutes of AF, whereas six horses remained in AF for more than 2.5 hours once an AF episode of 15 minutes or longer was obtained. Despite a reduction in AFR that started before drug infusion in the two early cardioverters and a difference in the mean AFR before drug infusion of 35 fpm between the two groups, no statistical significance was reached. The graphical behavior of AFR at the beginning of an acutely induced AF episode may, however, be an indicator of the length of the AF episode. In humans, it was found that AF episodes that terminated spontaneously within 5 minutes reached a lower peak fibrillation frequency than AF episodes of a longer duration.[7]

4.4 | Study limitations

The number of animals used in the present study was small and the statistical significance should be evaluated with caution. As a consequence, we have used graphical illustrations to support the findings of the study.

In our study set-up, we were unable to assess the conduction velocity during AF, which could have allowed us to determine the wave length and path length as well as the spatial excitable gap during AF. Consequently, we are only able to speculate on the mechanisms behind the antiarrhythmic effects of dofetilide and ranolazine alone and in combination in induced AF in horses. To asses this parameter, a more invasive study set-up is required involving implantation of several electrodes as previously done in, for example, dogs and goats[28,29] or by using a mapping system to secure a stable and similar position of electrodes in all horses.

If the half-life of ranolazine is short (currently unknown in horses), the dosing regimen, which had a duration of over 1.3 hours, may have been problematic. The desired plasma levels may not have been reached and a measure of plasma levels would, therefore, be very valuable. The doses infused have, however, previously been shown to cardiovert horses in AF faster than controls[3] and we, therefore, assume that the

plasma levels of ranolazine should have reached levels high enough to affect the AFR if this was the mode of action.

5 | CONCLUSION

Termination of AF after ranolazine administration followed a very rapid reduction in AFR immediately before rhythm restoration, which suggests that ranolazine AF-cardioverting mechanisms are not directly related to the slowing of the fundamental atrial fibrillatory frequency, limiting the ability of surface ECG to monitor ranolazine effects during AF. In contrast, however, our data support the use of surface ECG for monitoring the antiarrhythmic effects of dofetilide, which decreased AFR in a dose-dependent manner, even in low doses. Overall, this study has demonstrated the differences in the mode of action on AF between different antiarrhythmic drugs, detected using a noninvasive approach and our results further support the use of ECG-derived AFR monitoring for the assessment of antiarrhythmic drug effects during AF.

AUTHOR CONTRIBUTIONS

HC: Design, data analysis and interpretation, drafting article, approval of article, statistics, data collection, secured funding. EZH: Data analysis and interpretation, critical revision of article, approval of article, data collection. MMH: Design, critical revision of article, approval of article, data collection. MF: Critical revision of article, approval of article, data collection. JC: Data analysis and interpretation, critical revision of article, approval of article, statistics, secured funding. SP: Design, critical revision of article, approval of article. TJ: Design, critical revision of article, approval of article, secured funding. PPG: Data analysis and interpretation, critical revision of article, approval of article, statistics, secured funding. RB: Design, data analysis and interpretation, critical revision of article, approval of article.

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