Antiarrhythmic and electrophysiologic effects of flecainide on acutely induced atrial fibrillation in healthy horses

Haugaard, Maria Mathilde; Pehrson, S.; Carstensen, Helena; Flethøj, Mette; Hesselkilde, Eva Zander; Præstegaard, K. F.; Diness, J. G.; Grunnet, M.; Jespersen, Thomas; Buhl, Rikke

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Antiarrhythmic and Electrophysiologic Effects of Flecainide on Acutely Induced Atrial Fibrillation in Healthy Horses


Background: Only few pharmacologic compounds have been validated for treatment of atrial fibrillation (AF) in horses. Studies investigating the utility and safety of flecainide to treat AF in horses have produced conflicting results, and the antiarrhythmic mechanisms of flecainide are not fully understood.

Objectives: To study the potential of flecainide to terminate acutely induced AF of short duration (≥15 minutes), to examine flecainide-induced changes in AF duration and AF vulnerability, and to investigate the in vivo effects of flecainide on right atrial effective refractory period, AF cycle length, and ventricular depolarization and repolarization.

Animals: Nine Standardbred horses. Eight received flecainide, 3 were used as time-matched controls, 2 of which also received flecainide.

Methods: Prospective study. The antiarrhythmic and electrophysiologic effects of flecainide were based on 5 parameters: ability to terminate acute pacing-induced AF (≥15 minutes), and drug-induced changes in atrial effective refractory period, AF duration, AF vulnerability, and ventricular depolarization and repolarization times. Parameters were assessed at baseline and after flecainide by programmed electrical stimulation methods.

Results: Flecainide terminated all acutely induced AF episodes (n = 7); (AF duration, 21 ± 5 minutes) and significantly decreased the AF duration, but neither altered atrial effective refractory period nor AF vulnerability significantly. Ventricular repolarization time was prolonged between 8 and 20 minutes after initiation of flecainide infusion, but no ventricular arrhythmias were detected.

Conclusions and Clinical Importance: Flecainide had clear antiarrhythmic properties in terminating acute pacing-induced AF, but showed no protective properties against immediate reinduction of AF. Flecainide caused temporary prolongation in the ventricular repolarization, which may be a proarrhythmic effect.

Key words: Atrial electrophysiology; Equine; Pacing; Programmed electrical stimulation.

The utility and safety of flecainide to treat atrial fibrillation (AF) in horses has been evaluated in a few studies and with conflicting results.1–4 In acute experimentally induced AF studies, flecainide was reported to be a safe antiarrhythmic compound with high efficacy in restoring sinus rhythm (SR) in horses with AF of 15 minutes’ duration,1 but caused sudden death in 1 of 2 treated horses with 7 days of AF.4 Disappointing efficacy and questionable safety were found in horses with longer lasting AF, with evidence of electrical and mechanical remodeling.2–4

Atrial fibrillation is the most common clinically relevant arrhythmia affecting performance in athlete horses.5–8 Orally administered quinidine sulfate is the pharmacologic treatment of choice for restoring SR in horses regardless of the duration of AF,9 and has a fairly high efficacy (65–90%) in horses with no underlying cardiac disease.8 However, treatment frequently is terminated because of cardiac or noncardiac adverse effects or both.9,10 Transvenous electrical cardioversion (TVEC) is effective in restoring SR in horses without underlying cardiac disease with reported success rates of approximately 90%.11,12 However, this procedure requires specialized hospital facilities and equipment. Hence, alternative treatment options are desirable.

Atrial fibrillation is a self-sustaining arrhythmia characterized by rapid uncoordinated atrial activation and irregular ventricular contractions.13 The fundamental mechanisms underlying AF are still debated, but regardless of the initiating event, there is broad consensus that AF is maintained by rapid focal activation or by re-entrant wavelets of depolarization moving randomly across the atria.13–15 Flecainide is,
because of its sodium channel blocking properties, classified as a class IC antiarrhythmic drug. One of the prominent features of flecainide is slowing of the conduction velocity throughout the heart. However, the exact pharmacologic mechanisms are not fully understood. In vivo studies have produced conflicting results with regard to flecainide-induced changes in atrial effective refractory period (aERP). Flecainide may slow the atrial activation rate and eventually terminate AF by decreasing excitability, increasing the size and decreasing the number of re-entrant circuits, primarily by widening the temporal excitable gap (the difference between AF cycle length [AFCL] and aERP during AF), by changing the appearance of the rotating wavefront or some combination of these effects. In animal tachypacing models and in human AF patients, flecainide caused increased AFCL, decreased conduction velocity and caused a minor tachycardia-dependent increase in aERP. In horses, a marked flecainide-induced increase in AFCL has been reported, but the effects of flecainide on aERP, AF duration, and AF vulnerability have not yet been investigated.

The purpose of this study was to evaluate the potential of flecainide to terminate acutely induced AF of short duration and to assess the properties of flecainide on AF duration, and AF vulnerability in healthy horses. In addition, the acute electrophysiologic effects of flecainide on aERP, AFCL, and ventricular depolarization and repolarization time (QRS, QTc, and JTc) were investigated by standardized programmed stimulation methods.

Materials and Methods

Nine Standardbred horses were included: 3 geldings, 6 mares; age, 7.9 ± 3.9 years; and, body weight, 475 ± 47 kg (Table S2 in Data S1). Eight horses were included in a flecainide group and 3 horses in a control group. Two horses participated in both groups. The horses showed no signs of cardiovascular disease based on history, clinical examination, thorough cardiac auscultation, 24-hour ECG, and routine echocardiographic examination. The studies were approved by The Danish Animal Experiments Inspectorate (license number 2012-15-2934-00198) and performed in accordance with the Danish guidelines for animal experiments according to the European Commission Directive 86/609/EEC.

All experiments were performed in standing nonsedated horses restrained in a stock. Before catheterization, injection sites were aseptically prepared and locally anesthetized by using SC infiltration. In total, 3 12-gauge IV catheters were placed: 2 in the right and 1 in the left jugular vein. The left-sided catheter was used for drug administration, blood sampling, and routine access. The right-sided catheters were placed in the lower half of the vein approximately 10 cm apart and replaced by introducer sheaths. A multipolar steerable nonfixative electrode was introduced through each introducer sheath and advanced into the right atrium: 1 for atrial pacing, and the second for recording intra-atrial electrograms (aEGM). The electrodes were positioned in the atrium so that deflections on the aEGM appeared in close association with the beginning of the P wave on the 2 simultaneously recorded surface ECGs (S-ECG1 + S-ECG2), and pacing at 60 beats per minute (bpm) resulted in consistent atrial capture. Self-adhesive ECG electrodes were positioned as shown in Figure 1. S-ECG1 was optimized to show atrial activity (P wave lead), whereas S-ECG2 was a modified base-apex lead focusing on ventricular activity. In S-ECG1, the positive electrode was adjusted dorso-ventrally until the recorded P wave had the highest amplitude possible. Both intra-atrial and surface ECGs were
monitored during the experiments and stored for later analysis. Furthermore, an additional surface ECG (S-ECG3) identical to S-ECG2 was recorded with the Televet system and continued for 12–24 hours after the experiment. The experimental protocol of the study is demonstrated in Figure 2.

**Atrial Effective Refractory Period**

A constant pulse width of 2 milliseconds was used throughout the experiment. Measurements of aERP were conducted using twice baseline atrial pacing threshold. The aERP was determined once before and once after flecainide treatment at atrial pacing rates of 60, 75, 120, and 182 bpm, respectively. aERP was measured with 10 basic stimuli (S1) followed by an extra stimulus (S2) applied with 10 milliseconds increments, and was defined as the longest S1–S2 failing to elicit a new action potential. Every S1–S2 interval was applied multiple times (5–10 repetitions) at each pacing rate, and if intermittent atrial capture occurred, the S2 value below the one with ≥50% captures was defined as the aERP.

**Atrial Fibrillation Cycle Length**

Atrial fibrillation cycle length was measured as a surrogate for tissue refractoriness, and represents the time interval between consecutive atrial depolarizations. In the present study, AFCL was determined by manually analyzing 15-second sequences on the aEGM the last 15 seconds before the initiation of flecainide treatment and repeated in the last 15 seconds before AF termination.

**Induction of AF and Flecainide Treatment**

Atrial fibrillation was repeatedly induced using programmed electrical stimulation. A comprehensive induction protocol was used because the sensitivity of horses to induced AF has not been described previously, and because detailed information was needed to evaluate possible flecainide-induced changes in AF duration and AF vulnerability. A schematic outline of the induction protocol is illustrated in Figure 3. The duration of each tachypacing period was 2–6 seconds (Table S3 in Data S1). If the duration of an induced AF episode exceeded 5 minutes at any given induction setting, tachypacing was repeated 5 times. If episodes were shorter than 5 minutes, 10–15 inductions were made and mean AF duration was calculated. Comparisons (baseline versus post-flecainide) were based on results obtained at the baseline induction setting capable of inducing a mean AF duration >1 minute. In addition, the first single AF episode >1 minute in duration defined AF vulnerability (for vulnerability scoring, see Table S1 in Data S1). If only a few or short-lasting AF episodes

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Fig 2. The experimental protocol of the study based on ECG recordings. To emphasize that the same protocol was used before and after flecainide, identical ECG sequences are presented in B and E and in C and F. (A) Positioning of electrodes and atrial pacing threshold determination. (B) Atrial effective refractory period (aERP) measurements at multiple atrial pacing rates (60, 75, 120, and 182 bpm). (C) Atrial fibrillation (AF) duration and AF vulnerability measurements. AF was induced and the time to cardioversion at different induction settings (see Fig 3) was recorded. (D) If AF ≥ 15 minutes occurred horses were treated with flecainide (2 mg/kg IV) and cardioversion times recorded. (E) Repeated threshold determination and measurements of aERP at identical atrial pacing rates as before flecainide treatment. (F) Remeasuring of AF duration and AF vulnerability as described under (C). A black mark under a P wave indicates a second degree AV block. Arrow in (B) and (E): S2 stimuli associated with atrial capture. Arrow in (C) and (F): Tachypacing period leading to AF and subsequent spontaneous cardioversion.
were induced, the stimulation frequency was stepwise increased and at the final step (burst pacing at 3,000 bpm; ie, 50 Hz), the current was subsequently increased (Fig 3). Whenever an AF episode exceeded 15 minutes in duration, AF termination was attempted by IV infusion of flecainide [2 mg/kg] over 10 minutes according to previous recommendations. If no AF episodes ≥ 15 minutes occurred and spontaneous termination occurred repeatedly, horses were treated with flecainide while in SR. Seven horses were treated while in AF; 1 while in SR. After flecainide infusion, aERP measurements and AF induction protocols were repeated. Subsequently, the effects of flecainide on AF duration and AF vulnerability were assessed. Blood pressure (noninvasive) and blood samples were collected at baseline and at specific time points after flecainide treatment to evaluate flecainide-induced changes (for details see Data SI).

Time-Matched Control Studies

Three horses were assigned to participate in time-matched control studies. Two horses completed the control study after a flecainide wash-out period, whereas the last horse was included only in the control group. The control studies followed a protocol as described for the flecainide studies except that only isotonic saline was infused instead of flecainide. After infusion, the horses were monitored for spontaneous cardioversion for 30 minutes. If a horse was still in AF after 30 minutes, the experiment was ended and the horse was released from all equipment including intracardiac electrodes. However, S-ECG3 was left in position to capture the exact cardioversion time.

Analysis of ECG Intervals

QT intervals and QRS durations were manually analyzed on S-ECG2; JTe intervals were calculated as (QTc minus QRS). Representative ECG tracings illustrating QT and QRS measurements in SR and AF, as well as information regarding QRS/QTc-methods are provided in Data SI. Heart rate was calculated in the same sections as QRS and QT were measured.

Data Analysis

Results are presented as mean ± SD. Statistical analyses were performed by specialized software. All datasets were normally distributed allowing the use of parametric tests. However, logarithmic transformation of AF duration data was necessary to accomplish normal distribution. The combined effects of flecainide and atrial stimulation rates on aERP were examined by 2-way repeated-measures ANOVA followed by Bonferroni's posthoc test. A paired t-test was used to analyze flecainide-induced changes in AF duration, whereas a Wilcoxon matched pairs t-test was used to compare changes in AF vulnerability. Flecainide-induced changes in blood pressure (MAP), heart rate,
QRS, QTc, and JTc interval were analyzed by 1-way repeated ANOVA followed by Dunnett's posthoc test using the time point just before initiated infusion of flecainide as reference (Time – 1). P<.05 was considered statistically significant.

**Results**

To investigate the electrophysiologic effects of flecainide, we examined the atrial pacing threshold, AFCL, and aERP before and after infusion of flecainide. Flecainide did not alter the atrial pacing threshold compared to baseline (1.0 ± 0.9 versus 0.8 ± 0.5; Table S2 in Data S1). AFCL increased significantly before flecainide-induced AF termination compared to baseline with a mean increase of 35 ± 19% (P < .001; Table S2 in Data S1). However, treatment with flecainide did not result in statistically significant changes in aERP at any stimulation rate (Fig 4). Faster atrial pacing rate resulted in a decrease in aERP (P = .014; Table S3 in Data S1).

The antiarrhythmic potential of flecainide was studied by evaluating drug-induced termination time, changes in AF duration and AF vulnerability. The outcome of the induction protocol varied markedly among horses. However, AF episodes of variable duration were induced in all horses. Three of 8 horses were highly sensitive and developed AF episodes ≥15 minutes during baseline aERP measurements at an atrial pacing rate of 182 bpm (Table S3 in Data S1). In total, 7 of 8 horses developed AF ≥ 15 minutes during the baseline protocol and were treated with flecainide while in AF. Duration of the last AF episode before initiation of treatment was 21 ± 5 minutes (Table S3 in Data S1). In 1 horse, spontaneous AF termination occurred repeatedly, and only several short episodes of AF could be induced, and thus, this horse received flecainide while in SR. The 7 horses treated with flecainide while in AF, all cardioverted to SR during flecainide infusion with a termination time of 4.6 ± 2.5 minutes (Table S3 in Data S1).

Post-flecainide AF inductions were initiated 42 ± 13 minutes and ended 72 ± 24 minutes after completed infusion. Plasma concentrations of flecainide (ng/mL) 1 minute to 24 hours after completed infusion are illustrated in Figure 5 and plasma half-life ($T_{1/2}$) values are presented in Table S4 in Data S1. The flecainide-induced changes in AF duration and AF vulnerability are shown in Figure 6A,B. All horses completed the post-treatment aERP protocol without developing any AF episodes ≥15 minutes. Flecainide significantly shortened AF duration ($P = .003$; Fig 6A, Table S3 in Data S1), but had no statistically significant effect on AF vulnerability ($P = .136$; Fig 6B, Table S3 in Data S1).

Baseline MAP was 78 ± 12 mmHg. Flecainide did not significantly change MAP during a 60-minute period after completion of infusion compared to baseline (Fig 7).

To evaluate flecainide-induced changes in the ventricular conduction pattern, we examined changes in HR, and QRS, QTc, and JTc intervals. QRS, QTc, and JTc were prolonged between 8 and 30 minutes, 6 and 25 minutes, and 8 and 20 minutes after initiation of flecainide infusion, respectively (Fig 8A,C,D). HR was calculated over 20 ± 1.8 beats and was stable throughout the experiments (Fig 8B). No ventricular arrhythmias were detected during infusion and in a 90-minute period after flecainide treatment.

**Control Studies**

To measure AFCL at comparable time points in the flecainide and in control groups, AFCL after saline infusion was measured at the previously recorded time of cardioversion from AF to SR after flecainide infusion (4.6 minute after starting infusion = during infusion).
Baseline atrial pacing threshold, pre-saline AF duration, pre-saline AFCL, AFCL at 4.6 minutes after saline infusion was started, and baseline aERP at 60 and 182 bpm in the control experiments are presented in Table S5 in Data S1. All 3 horses developed AF episodes ≥15 min during the baseline protocol with a mean AF duration of 22 ± 5 min before saline infusion was started. AF was not terminated by saline infusion, and spontaneous termination time was 5.8 ± 3.2 hours (Table S5 in Data S1).

Discussion

This study was designed to investigate flecainide-induced effects on atrial electrophysiology in horses with acutely induced AF of short duration. We assessed the antiarrhythmic potential of flecainide by its cardioversion potential and effects on AF duration and AF vulnerability. The in vivo electrophysiologic profile of flecainide was evaluated by changes in aERP, AFCL, QRS, QTc, and JTc.

Flecainide is a clinically important compound in human patients with paroxysmal AF, but for safety reasons, candidates for treatment are carefully selected and the use of flecainide is restricted to patients without structural heart disease and with preserved left ventricular function.31,32 When these criteria are fulfilled, treatment with flecainide demonstrates cardioversion rates of 57–92% in human patients with AF of short duration.32 Flecainide terminated all AF episodes in the present study with a termination time comparable to that observed in previous studies,1,27 and at the evaluated stimulation rates, the presented baseline values of aERP in horses were consistent with those reported by others.4,33 Our study identified no flecainide-induced changes in aERP, indicating that flecainide did not have an influence on atrial repolarization at the chosen stimulation rates. Depending on the model, flecainide has been reported to have variable effects on aERP in vivo.16–20 In humans, some reported a slight but not significant flecainide-induced increase in aERP, whereas others reported significant and use-dependent increases.20,34 A flecainide-induced reduction in aERP accommodation to atrial activation rates, which may be important in suppressing AF, has been demonstrated by others.18,34–36 However, this mechanism of rate-dependency and increased drug action at rapid atrial activation rates could not be proven by the present study. Still, despite the lack of aERP prolongation at the atrial rates measured in the present study, we cannot rule out possible increases in...
aERP during the rapid rates typical of AF. Our observations are in agreement with results from studies conducted in goats where aERP was unaffected, AFCL increased, and AF vulnerability unchanged by flecainide.17,24 The variable flecainide-induced changes in aERP may be related to species differences and species-specific atrial ion channel composition.

Atrial fibrillation cycle length shortens when AF is maintained4,27,37,38 prolongs immediately before spontaneous or drug-induced termination18 and might provide a risk assessment for AF recurrence.18 The observed flecainide-induced increase in AFCL in the present study correlated well with the individual termination time (Tables S2, S3 in Data S1). However, in 2 horses the termination times were strikingly short and correlated with very limited increases in AFCL, indicating a questionable drug-related termination of AF. However, in the majority of horses, the flecainide-induced increase in AFCL corresponded well with results reported by others.7,17,24,27 A single study investigating flecainide-induced changes in AFCL in horses with spontaneous AF reported comparable increases, nevertheless none of the treated horses cardioverted in response to treatment.7 These conflicting results can most likely be explained by the difference in AF duration and hence differences in the degree of electrical remodeling. Van Loon et al. reported baseline AFCL values approximately 30 milliseconds shorter than ours, which supports the assumption of extensive electrical remodeling in the previous study.2 These findings emphasize the notion that AF should be terminated as quickly as possible to prevent electrophysiologic remodeling which will potentially drive the tissue into the cycle of “AF begets AF.”

In the present study, we found a flecainide-induced decrease in induced AF duration, but no significant influence on AF vulnerability. No previous studies that were methodically comparable with regard to flecainide-induced changes in AF duration and AF vulnerability were found in the literature. However, a study in dogs, investigating flecainide’s properties against AF-promoted electrical remodeling identified no benefit of flecainide on either of the 2 parameters, and a goat model showed 100% reinducibility of AF after cardioversion with flecainide.24,39 The results from the present study suggest that flecainide is effective in terminating acutely induced AF of short duration, has a positive diminishing effect on AF duration but does not protect against immediate reinduction of induced AF in healthy horses.

The safety profile of flecainide was evaluated by flecainide-induced changes in QRS and QTc.
durations were measured as a surrogate for the effect of flecainide on ventricular conduction velocity, and our results were in agreement with results reported by others.1,2 Because of sodium channel blockade, and hence depressant effects on cardiac conduction, a flecainide-induced widening of QRS was expected.20,40 In humans, recommendations are that flecainide treatment should be discontinued if QRS prolongation exceeds 25% of baseline.36,41 The same limit of 25% is used in equine medicine to suggest quinidine toxicity,32 but such recommendations are not available for flecainide. However, because both quinidine and flecainide are sodium channel blockers, it might be reasonable to expect a similar limit to indicate flecainide toxicity. Toxicity was not indicated in any of the horses included in the present study because the maximum widening of QRS did not exceed 25% at any time during the experiments. However, the effect of flecainide is use-dependent, and therefore flecainide-induced changes in QRS may be increased at higher heart rates than reported in the present study. To investigate whether flecainide affected ventricular repolarization, we evaluated changes in QTc and JTc intervals. In a previous study of horses, no flecainide-induced changes in QT intervals were reported.1 In our study, QTc was significantly prolonged between 6 and 25 minutes after flecainide infusion was started. To obtain a more specific ventricular repolarization time, JTc intervals were calculated, and in contrast to what has been described in humans,20 flecainide temporally prolonged JTc in horses. The present study however did not identify any potentially dangerous ventricular arrhythmias, which is in agreement with results reported by other studies.1,3 However, 3 cases of flecainide-induced wide QRS-tachycardia have been reported in horses2,4 of which 1 episode led to ventricular fibrillation and sudden cardiac death. Combined with the altered ventricular repolarization observed in the present study, it is important to recognize the potential proarrhythmic properties of flecainide.

Acutely induced AF in horses is a unique technique to mimic AF. The present study reports results on pacing-induced short episodes of AF in healthy horses and the applicability to longer lasting spontaneous AF is therefore uncertain. Two studies investigated the antiarrhythmic potential and safety of flecainide on longer lasting naturally occurring AF in horses and reported disappointing results.2–4 Only 1 of 14 attempts to terminate naturally occurring AF restored SR. In addition, the horse that responded positively to treatment had recent-onset AF of only 12 days’ duration. Another study used flecainide to cardiovert persistent burst pacing-induced AF of 7 days’ duration. Only 1 of 2 horses regained SR.4 Such observations support the theory of AF duration being an extremely important parameter when estimating the potential efficacy of flecainide, and that treatment with antiarrhythmic drugs always carries a risk of inducing devastating ventricular arrhythmias.

In conclusion, flecainide had clear antiarrhythmic effects terminating acute pacing-induced AF of short duration (21 minutes) and resulted in decreased AF duration in healthy horses, but flecainide had no protective properties against immediate reinduction of AF and caused temporary prolongation in ventricular repolarization, which may be a potentially dangerous proarrhythmic effect.

Footnotes

a Carbocain 20 mg/kg, AstraZeneca, Copenhagen, DK
b Intralron 2 (PTE), 12G, Vycon, Wiltshire, UK
c One Piece/Tuohy-Borst Catheter Introducer with Integral Hemostasis Valve, SF, Argon Medical Devices, Holte, DK
d Inquiry Steerable Diagnostic Catheter, 6F/110 cm, St. Jude Medical, Inc, Glostrup, DK
e Kruuse, Langeskov, DK
f LabChart 7 Pro, ADInstruments, Oxford, UK
g Terumo, 10 mg/mL, Meda AS, Allerød, DK
h PM-9000Vet, Portable Veterinary Monitor, Mindray, tail-cuff system
i Venosafe Lithium Heparin tubes, 9 mL, Terumo, SE
j GraphPad Prism 5 Software, San Diego, CA

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Conflict of Interest Declaration: The authors disclose no conflict of interest.

Off-label Antimicrobial Declaration: The authors declare no off-label use of antimicrobials.

References


Supporting Information
Additional Supporting Information may be found in the online version of this article:

Data S1. Materials and Methods.