

Pathophysiology and treatment of atrial fibrillation in horses

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LIST OF ABBREVIATIONS

a	at maximal atrial contraction
AD	amiodarone
AERP	atrial effective refractory period
AF	atrial fibrillation
AFCL	atrial fibrillation cycle length
Ao	aorta
AOD	aortic diameter
AODV	aortic diameter at valvular level
AUC	area under the curve
CL	cycle length
Cl	clearance
C_{\max}	maximum plasma concentration
C_p	plasma concentration
CV	conduction velocity
d	at the end of ventricular diastole
DAD	desethylamiodarone
ECG	electrocardiogram
F	absolute bioavailability
FAC	fractional area change
FS	fractional shortening
HPLC	high-performance liquid chromatography
HR	heart rate
IRAF	immediate recurrence of atrial fibrillation
IV	intravenous
k	elimination rate constant
LA	left atrium
LAA	left atrial area
LA_{sx}	left atrial short-axis
LAD	left atrial internal diameter
LAS	left atrial surface
LOD	limit of detection
LOQ	limit of quantification
LPA	left pulmonary artery
LVIDd	left ventricular internal diameter in diastole
LVIDs	left ventricular internal diameter in systole
p	at the onset of the P wave
PCL	pacing cycle length
PO	per os

<i>R</i>	rate of infusion
RA	right atrium
RPA	right pulmonary artery
RV	right ventricle
s	at the end of the ventricular systole
SF	French saddle horse
SVT	supraventricular tachycardia
<i>t</i>	time
$t_{1/2}$	half-life
$t_{1/2el}$	terminal elimination half-life
T_{max}	time of C_{max}
TV	tricuspid valve
V_d	volume of distribution
VERP	ventricular effective refractory period
V_{max}	maximum upstroke velocity of the action potential
V_p	volume of distribution of the central compartment
WL	wavelength
QS	quinidine sulphate

Atrial fibrillation is the most important arrhythmia in horses as it develops relatively easy and because it commonly affects athletic ability. When no other predisposing cardiac pathology is present, prognosis after conversion to sinus rhythm is good because horses generally regain their full athletic potential. Therefore, in competition horses, treatment by ‘rhythm control’ is indicated and worthwhile. Quinidine sulphate is the gold standard treatment for this arrhythmia in horses. However, the drug becomes more difficult to obtain in several countries. Nowadays, quinidine is almost completely abandoned in human medicine because many, less toxic but effective alternatives are available.

For the first section of this thesis electrical and contractile remodeling and reverse remodeling associated with acute and chronic atrial fibrillation were studied to better understand the pathophysiology of this arrhythmia in horses. In the second and third section, the results of new pharmacological treatments and a non-pharmacological therapy for horses with AF are described.

1. Atrial fibrillation

With a prevalence of around 2.5% (based on patient data of 2477 horses) (Else and Holmes, 1971) and an important impact on athletic performance, atrial fibrillation (AF) represents the most important supraventricular arrhythmia in horses. AF is characterized by disorganised atrial electrical activity and a loss of atrial contribution to ventricular filling. In human patients, AF prevalence is around 1-2% and increases with age: the median age of patients with AF is 75 years and whereby 84% of the patients with AF are older than 65 years (Benjamin et al. 1994; Feinberg et al., 1995; Benjamin et al., 1998; Go et al., 2001). In horses, AF shows no clear relationship with age.

2. Classification of AF

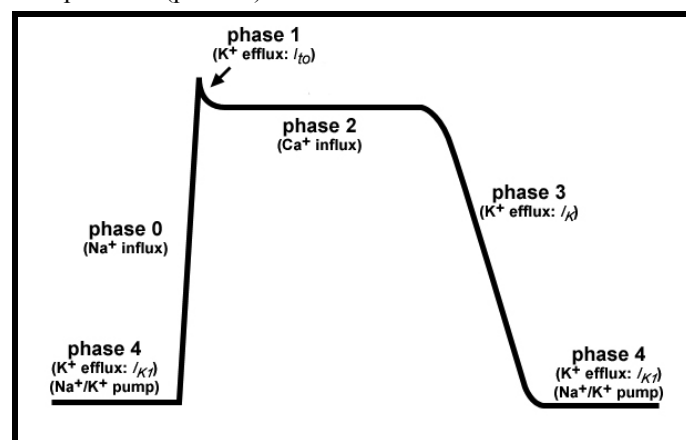
AF can be classified based upon the presence of predisposing disease or based upon AF duration. AF can be found as a result of an underlying disease such as mitral regurgitation, atrial dilatation, or it can be found as ‘lone’ (uncomplicated, primary) AF. Based upon AF duration, human patients are subdivided in (1) paroxysmal AF, when AF terminates spontaneously within hours or days, in (2) persistent AF, when it continues for a longer period or when it requires a pharmacological or electrical cardioversion, and in (3) permanent AF, when AF continues with or without treatment attempts (Allesie et al., 2001; Crijns et al., 1999; Gallagher and Camm, 1998; Fuster et al., 2006). Paroxysmal and lone AF tends to be seen more often in younger people. Epidemiological data suggest that 29% of the patients with paroxysmal AF without underlying heart disease progressed to permanent AF within 15-30 years (Jahangir et al., 2007). The prevalence of paroxysmal AF was reported to be 1.39% in slowly finishing and non-finishing race horses (Ohmura et al., 2003).

3. Initiation of AF

The normal action potential of a myocardial cell shows 4 different phases (Figure 1).

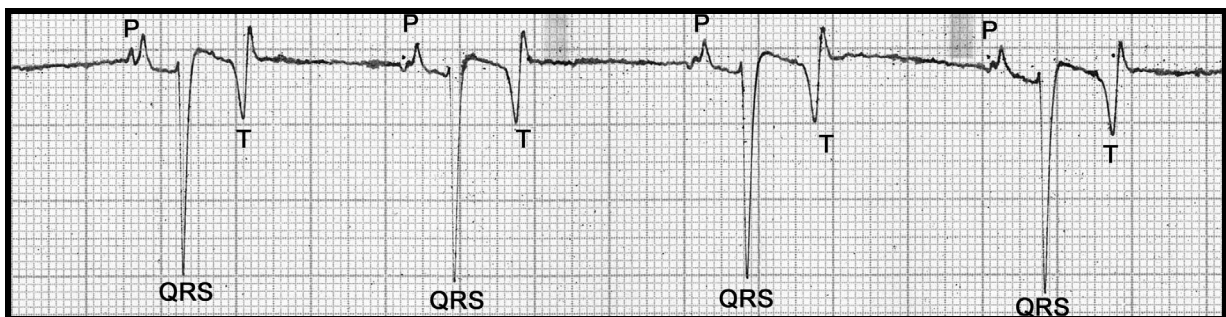
Phase 0 is the rapid depolarization phase. The slope of phase 0 represents the maximum upstroke velocity of the action potential and is known as V_{max} . During this phase the fast Na^+ -channels open and a rapid increase in the membrane conductance for Na^+ appears. The Na^+ -channels contain different gates which gradually open. They are totally closed at the onset of phase 0 and totally opened at the end of phase 0. During *phase 1*, the cell partially repolarizes through a transient outward K^+ current (I_{to}), inactivation of which produces a notch in the action potential. *Phase 2*, the “plateau phase”, is sustained by an inward L-type calcium current. A series of K^+ current that activate in a time-dependent way, the delayed rectifiers (I_K), lead to cellular repolarization, *phase 3*. The delayed rectifiers consist of 3 components: an ultrarapid component (I_{Kur}), a rapid component (I_{Kr}) and a slow component (I_{Ks}). The balance between the inward movement of Ca^{2+} through the L-type Ca^{2+} -channels and the outward movement of K^+ through the delayed rectifiers determines the duration of the action potential: increased inward current prolongs the action potential and increased outward current abbreviates it. At the end of phase 3, when the membrane potential is restored, the delayed rectifiers close again. During *phase 4*, the negative resting membrane potential is maintained by the inwardly rectifying K^+ current (I_{K1}) and the Na^+ - K^+ pump. The latter transports 3 Na^+ outward the cell for 2 K^+ ions inward, thereby generating a net outward movement of positive charges. This phase of the action potential is associated with the diastole.

Figure 1. The different phases (0-4) during a ventricular action potential. At rest (phase 4) resting membrane potential is between 70 and -85 mV. A rapid sodium influx during phase 0 makes the membrane potential positive. Due to a rapid inactivation of the sodium channels and an efflux of potassium (I_{to}) the cell begins to repolarize (phase1). However, repolarization is interrupted by an influx of calcium and partial closure of potassium channels (phase 2) resulting in a plateau phase in the action potential. Due to re-opening of the potassium channels (I_K) and closing of the calcium channels the cell further repolarizes (phase 3) and eventually reaches its resting membrane potential (phase 4).



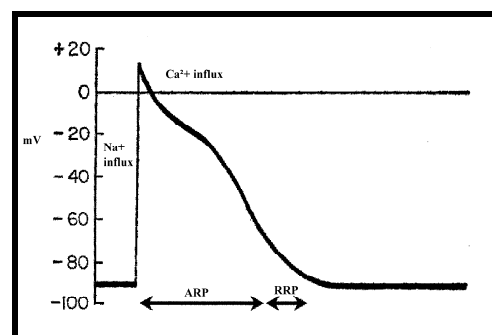
Some myocardial cells show a spontaneous depolarization. These pacemaker cells are located at the sinus node, which is located at the junction of the right atrium and vena cava cranialis. Under normal circumstances during sinus rhythm, these cells repetitively produce an action potential at a specific rate. The electrical impulse depolarizes right and left atrium, which results in a P wave on a surface electrocardiogram (ECG) (Figure 2). Subsequently, the impulse is slowly conducted through the atrioventricular (AV) node, which is the only electrical connection between atria and ventricles. When the impulse leaves the AV node, a rapid conduction via the His- and Purkinje-system depolarizes both ventricles. The latter results in a QRS complex on the surface ECG (Figure 2). After depolarization, the repolarization of the ventricles produces the T wave on the surface ECG.

Figure 2. The surface ECG of a horse in sinus rhythm.



During the depolarization-repolarization process, myocardial cells are unresponsive or refractory, to further excitation. This refractory period can be subdivided in the absolute refractory period and relative refractory period (Figure 3). During the absolute refractory period the myocardial cell is totally unresponsive to external stimuli whatever the strength. During the relative refractory period the myocardial cell is only responsive to external stimuli with a higher than normal amount of energy.

Figure 3. The normal atrial action potential duration and the absolute (ARP) and relative refractory period (RRP) of the myocardium.



Human and experimental data

Different theories have been proposed to explain the mechanism of AF primarily based upon ectopic foci, the presence of multiple re-entry waves, the leading circle concept or the presence of rotors and spiral waves.

3.1 Ectopic foci

One of the oldest theories about the initiation of AF, described by Lewis and Schleiter in 1912, is based on the presence of ectopic foci. These ectopic foci are regions in the atria which are able to deliver premature electrical impulses. With atrial mapping techniques, the presence and localisation of such ectopic foci has been demonstrated with most of the foci being located in the pulmonary veins or vena cava superior. Other locations are the right atrium, left atrium, vein of Marshall, crista terminalis and the coronary sinus (Shah et al., 1999).

In 95% of the cases the foci are typically localised in the pulmonary veins. Sleeves of myocardial tissue extending into the pulmonary veins are supposed to be the cause, because these sleeves have the same embryonic origin as the conduction system, which could explain their arrhythmogenic nature (Blom et al., 1999).

Multiple ectopic foci as well as single foci from individual heart fibres, firing at a high rate, may give rise to the uncoordinated electrical activity that initiates or even maintains AF (Engelman, 1896; Winterberg, 1906).

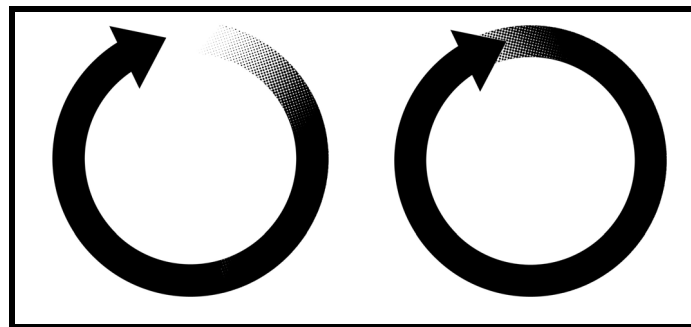
3.2 Re-entry and multiple waves

Re-entry is caused by abnormal impulse propagation and it can be initiated when an extra stimulus is delivered near a region with different electrophysiological properties, i.e. a different refractoriness and conduction velocity. The premature impulse will conduct through the cells with a short refractory period (these cells have recovered more rapidly) but will be blocked by the cells with a longer refractory period. As such, ‘unidirectional block’ occurs. By the time the impulse reaches the tissue with the longer refractory period, this tissue has fully recovered and the impulse can loop back to its origin to excite the tissue with the short refractory period again. As such a re-entry loop is formed, this can, under the right circumstances, continue to loop around and act as a continuous source of electrical activity.

The size or path length (PL) of the loop is determined by the length of the depolarisation wave, the wavelength (WL), and the amount of excitable tissue in between the ‘head’ and ‘tail’ of the depolarisation wave, the so called excitable gap (EG). As such $PL=WL+EG$. The WL depends on the conduction velocity (CV) and the atrial effective refractory period of those cells (AERP). As such $WL=CV \cdot AERP$ (Moe, 1962).

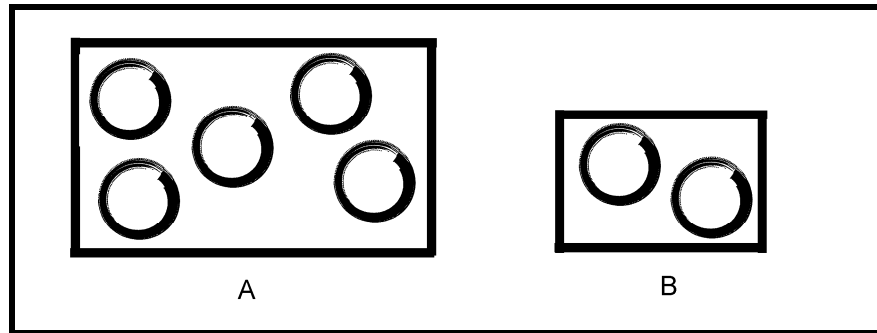
It is obvious that due to an increase in conduction velocity and/or an increase in refractoriness the excitable gap will become smaller. If sufficient reduction in the excitable gap occurs, the head of depolarisation wave will hit its own tail. This means that it will be blocked by refractory tissue which would terminate the re-entry loop (Figure 4).

Figure 4. At the end of the depolarization wave (black arrow), tissue gradually regains excitability (grey area) until it becomes fully excitable again (white area). The excitable gap (white area) allows the depolarization wave to continue its looping movement. The distance the wave travels during the duration of the refractory period is the wavelength (black + grey area). An increase in refractory period (right image) might terminate the loop as the ‘head’ of the wave hits refractory tissue.



During atrial fibrillation multiple re-entry circuits are thought to be present simultaneously, all meandering chaotically through the atria. The more wavelets that can coexist in the atria, the more stable AF becomes. An increase in atrial size or a decrease in wavelength (for example by a decrease in refractory period) increases the number of wavelets and thus promotes AF (Figure 5). It has been suggested that at least 6 re-entry circuits must coexist in order for AF to become self-sustained (Allessie et al., 1985).

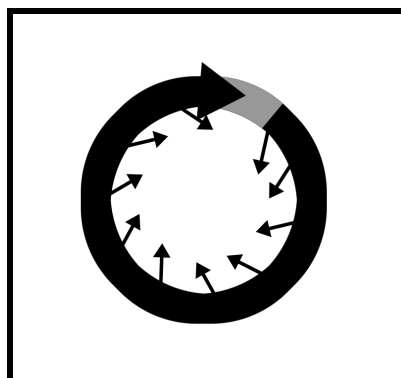
Figure 5. A large atrium (A) can contain more wavelets than a small atrium (B).



3.3 The leading circle concept

The theory of the leading circle is based on an impulse that makes a circular movement in a certain direction due to unidirectional block (Figure 6) (Allessie et al., 1973). The length of this circle is approximately equal to the wavelength of the impulse ($WL=CV \cdot RP$), i.e the distance the impulse travels during the refractory period. The excitable gap is very small. The impulse will also conduct to the centre of the circle (Figure 6) maintaining the centre constantly in a refractory state. The leading circle acts as a continuous source of electrical pulses that conduct centrifugally, activating the rest of the myocardium.

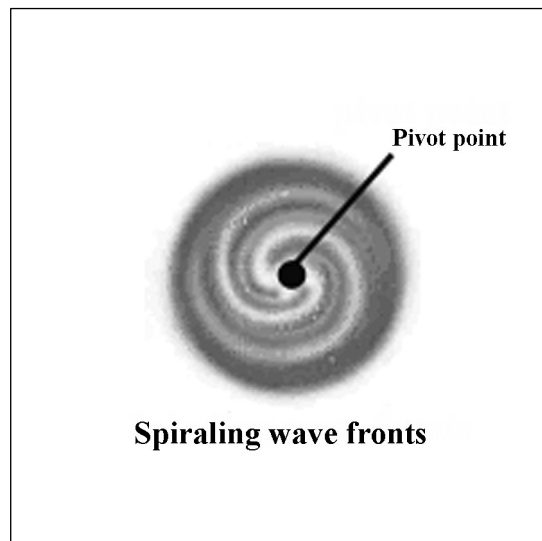
Figure 6. The leading circle (large black arrow) is a re-entry wave that follows the smallest possible pathway, whereby the excitable gap is very small. Centripetal waves originating from this leading circle constantly maintain the central core in a refractory state.



3.4 Rotors and spiral waves

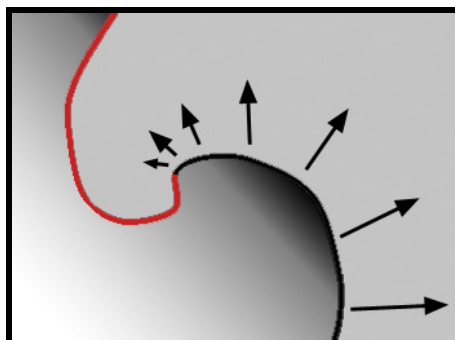
Another possible mechanism of AF is based on re-entry by one or more rotors that produce spiral waves (Figure 7), thereby acting as a continuous source of electrical activity (Jalife et al., 2002).

Figure 7. A rotor is a stable rotating reaction around a pivot point which gives rise to spiralling wave fronts.



A rotor is a depolarisation wave that continuously turns around a small pivot point in the centre of the spiral, whereby the depolarisation wave propagates away from this centre (Comtois et al., 2005). The front of the central depolarisation wave is curved (convex) (Figure 8). Due to differences in excitability, whereby the most central area shows lower excitability (small central arrow) than the rest of the curved depolarization front (larger arrows), a twisting movement is made (Cosio and Delpon, 2002). The tissue excitability determines the size of the pivot point and thus the spiral wave. In excitable tissue the rotor will easily turn around a small pivot point. However, due to a decreased excitability of the myocardium, the centre of the rotor fails to conduct in a small circle. The rotor needs more space to proceed and turns around in a larger circle, whereby it is more likely that the rotor and spiral waves terminate.

Figure 8. Because the front of the rotor (black line) is curved and because the most central part shows lower excitability (small arrow) than the more outer parts (larger arrows), the rotor makes a turning movement, producing a spiral wave. The radius of rotation is determined by the excitability of the tissue. The repolarization front is shown in red.

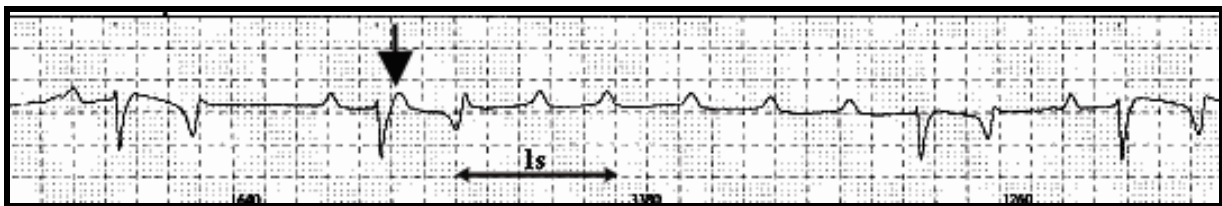


The presence of more rotors in both atria have been described and optical mapping techniques have shown that most dominant rotors are localised at or near the pulmonary veins and the posterior left atrial (LA) wall. Intermittent or permanent high frequency activation initiated by the rotors in the LA, results in conduction towards the right atrium and induces and maintains AF (Jalife, 2003).

Equine data

Similar to human medicine, AF is thought to be initiated by one or more premature beats coming from an ectopic focus but the exact localisation of ectopy has never been documented in horses. Whether the mechanism of AF is ectopy, re-entry or a rotor is also not known. Clinical data have demonstrated increased ectopy in horses with an increased susceptibility for AF (Figure 9).

Figure 9. The resting surface ECG of this horse shows a bout of rapid atrial ectopy (the start is indicated by the arrow). Within a few weeks this horse had developed persistent AF.



4. Perpetuation of AF

Human and experimental data

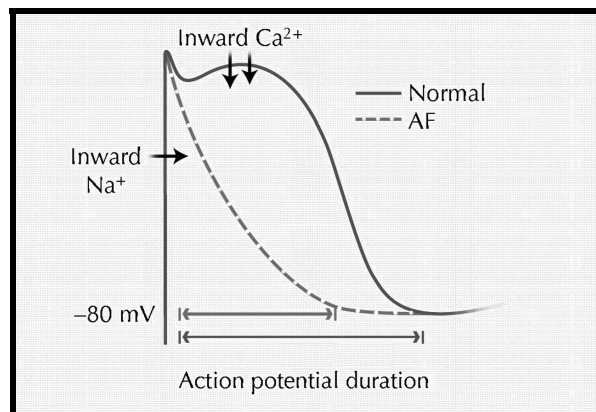
Besides the initiation of AF, the substrate, i.e. the atria, must be able to allow perpetuation of the arrhythmia. As already mentioned, atrial size, atrial electrical characteristics and electrolyte balance, alterations in autonomic tone and the presence of structural lesions have a major impact on AF stability. In addition, AF *per se* induces important electrical, contractile and structural remodeling that further promote the perpetuation of the arrhythmia ('domestication' of AF) (Wijffels et al., 1995).

4.1 Electrical remodeling

When a cardiac cell is activated, the depolarization time, identified by an inward flux of positively charged ions (Na^+ -influx), is followed by a membrane potential plateau which is associated with an L-type inward calcium current. Both parameters, but especially the membrane potential plateau, contribute to the duration, and thus refractory period, of the action potential.

Rapid rates, as observed in atrial fibrillation, increase the myocyte calcium load because of the rapid and repetitive inward calcium flow. Because high calcium concentrations can be toxic, the cell will protect it self by a down regulation of the L-type calcium channel. This process reduces the inward calcium current which results in a shortening of the action potential and thus a shortened refractory period (Figure 10).

Figure 10. Due to atrial fibrillation the duration of the action potential and therefore the refractory period decrease as a result of a decreased calcium current during the plateau phase.



The reduction in refractory period implies a shortening in re-entry wavelength whereby more wavelets can coexist in the same atrium, which promotes the self-sustainability of AF.

In humans, goats, dogs and ponies, research has illustrated that shortening of the atrial refractory period develops within the first days of AF (Morillo et al., 1995; van Loon et al., 2001; Wijffels et al., 1995).

4.2 Contractile remodeling

Intracellular calcium (Ca^{2+}) concentration plays a major role in the contractile activity of the myocardial cell. During the contraction phase, Ca^{2+} is pumped into the cell followed by an outward Ca^{2+} current during the relaxation phase. When depolarisation rate increases

permanently, Ca^{2+} cannot properly be pumped out of the cell resulting in changes in the Ca^{2+} induced Ca^{2+} release and contractile function. These changes would then be responsible for the contractile deficit of the myocyte during AF. Research has demonstrated that such a reduction in atrial contractile function appears after a few days of AF (Courtemanche et al., 1998; Allessie et al., 2002). In addition, in human patients and dogs, prolonged AF is associated with myolysis which further reduces atrial contractile function. The atrial contractile dysfunction contributes to impaired atrial transport function and the occurrence of atrial thrombi (Stoddard, 2000).

Equine data

In horses, atrial contractile function can be assessed by cardiac ultrasound (Schwarzwald et al., 2007b). Atrial contractile dysfunction was found after successful cardioversion of AF in horses (Schwarzwald et al., 2007c). However, from that study, the influence of residual treatment effects or the presence of underlying primary myopathy could not be ruled out. In an experimental AF model in healthy ponies and in the absence of drug interference, atrial contractile dysfunction was clearly shown as a result of maintained AF (van Loon, 2001).

4.3 Structural remodeling

Histological changes that appear as a result of AF include cellular hypertrophy, glycogen accumulation, increased interstitial fibrosis, disruption in the sarcoplasmic reticulum, redistribution of the gap junctions and apoptosis (Ausma et al., 1997; Elvan et al., 1997; Van der Velden et al., 1998).

Equine data

Electrical and contractile remodeling during an induced chronic AF protocol in ponies indicated rapid electrical remodeling and contractile remodeling and a slower occurring dilatation of the left atrium (van Loon et al., 2001).

Data on structural remodeling in ponies or horses are not available yet.

5. Effect of AF on ventricular function

Human data

Due to the high atrial rate, AF is usually associated with an increase in heart rate. The reduction in diastolic time reduces ventricular filling and impairs cardiac function. Ventricular filling is further reduced because of the atrial contractile dysfunction. Especially at higher rates, under normal circumstances, the atrial contraction accounts for up to 15% of ventricular filling (Veng-Kin and Sagawa, 1979).

Equine data

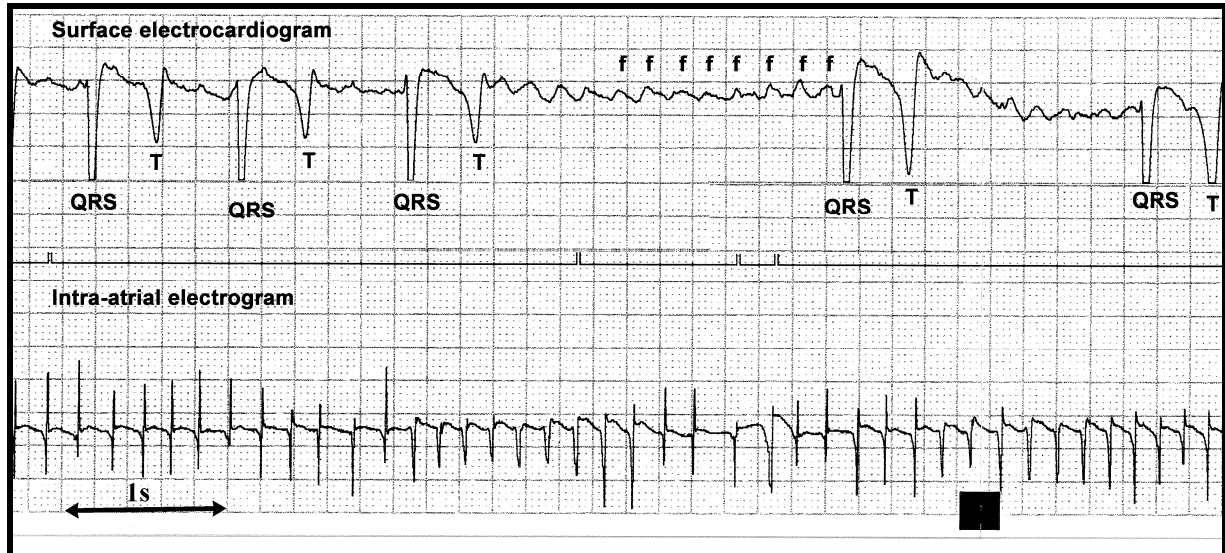
In horses, the atrial activation rate during AF is about 250-450/min. Nevertheless, because of the high vagal tone in horses, the atrioventricular (AV) node blocks most of these pulses. As a result the final ventricular rate at rest is normal, at least if no other significant cardiac disease is present. As the normal heart rate allows sufficient passive ventricular filling and thus a sufficient cardiac function (Muir and McGuirk, 1984), the horse shows no symptoms at rest. However during exercise, the sympathetic tone supersedes the vagal tone whereby conduction through the AV node is suddenly facilitated, resulting in a disproportionate tachycardia. Both the loss of atrial contraction and the extreme increase in heart rate during exercise result in a reduced cardiac function and exercise intolerance. However, the latter is predominantly seen at higher performance levels as sufficient compensation usually occurs at lower exercise intensity.

6. Diagnosis of AF in horses

AF is characterized by a totally disorganized atrial electrical activation without effective atrial contraction. It can be detected by auscultation as an ‘irregularly irregular’ heart beat. Final diagnosis is made by an electrocardiogram that shows irregular baseline undulations (‘f waves’), absence of P waves, normal QRS morphology and irregular RR intervals. The fibrillation ‘frequency’ or ‘rate’ is difficult to derive from the surface ECG because the f waves represent a summation of all individual atrial wavelets. Moreover, the f waves on the surface ECG continuously change in amplitude and morphology. The local frequency of

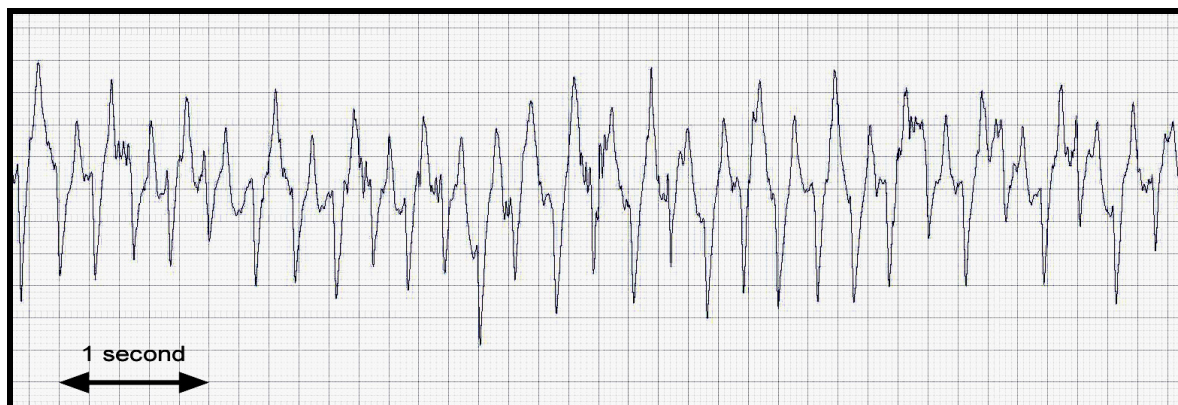
fibrillation can be accurately determined by recording an intra-atrial electrogram and is usually between 250 and 450 per minute when measured in the right atrium (Figure 11).

Figure 11. Surface ECG and simultaneous intra-atrial recording from a horse with atrial fibrillation at rest. f waves are found, P waves are absent, QRS morphology is normal and RR intervals are irregular.



As mentioned before, high vagal tone maintains a normal, although irregular, heart rate at rest. During exercise, however, heart rates of more than 280 beats per minute are commonly observed in otherwise healthy horses (Figure 12) (Deegen, 1986; Deegen and Buttenkotter, 1976; Maier-Bock and Ehrlein, 1978).

Figure 12. Exercise electrocardiogram from a horse with atrial fibrillation. An irregular R-R interval is detectable and instantaneous heart rate reaches 258 beats per minute.



Diagnosis of paroxysmal AF as a cause of poor performance is hampered by the fact that the arrhythmia suddenly develops during strenuous exercise and disappears shortly thereafter. As such, examination of the horse before and after a race might reveal no abnormalities. In order to diagnose paroxysmal AF, continuous ECG recordings during exercise are mandatory.

7. Symptoms of AF in horses

Symptoms depend on the degree of underlying heart disease and the exercise demanded from the patient. In breeding or pleasure horses, AF is usually an incidental finding unless concomitant congestive heart failure would be present.

In maximal or submaximal performance, exercise intolerance is always observed (Mitten, 1996). Especially in Thoroughbreds paroxysmal AF has been found during racing and was associated with a sudden decrease in performance (Amada and Kurita, 1975; Hiraga and Kubo, 1999; Holmes et al., 1986; Miller et al., 1992; Rose and Davis, 1977). Such horses may suddenly pull-up and show signs of lung oedema, epistaxis, incoordination and even collapse, presumably because of a decreased cardiac pumping function and an increased atrial pressure with a reduction in pulmonary circulation.

8. Prognosis for horses with AF

Prognosis for life is excellent if no other cardiac disease is present. Horses with lone AF and with an AF duration of less than 2 months generally respond well to treatment in more than 85% of the cases and even return to their previous athletic performance (Reef et al., 1988). However, AF recurrence rate in these horses varies from 15 to 30% and horses with more longstanding AF are more difficult to treat (Deegen and Buntenkotter, 1976; Deem and Fregin, 1982; Reef et al., 1988).

In horses with mild to moderate underlying cardiac disease, such as atrial dilatation due to mitral regurgitation, conversion rate is lower and recurrence rate is higher.

9. Treatment

Because prognosis is rather good in horses without underlying heart disease, treatment is generally recommended if these animals are further intended to perform.

Pharmacological as well as non-pharmacological treatment of the arrhythmia has been described.

9.1 Pharmacological treatment

General

In human patients, anti-arrhythmic drugs are administered to decrease heart rate ('rate control') or to restore the normal heart rhythm ('rhythm control') (Herzog et al., 2005; King et al., 2002). The 'Vaughan-Williams' classification is one of the most widely used classifications for anti-arrhythmic agents. Although many anti-arrhythmic drugs have multiple action mechanisms, they are classified based on the primary mechanism of anti-arrhythmic effect and the effect on the different phases during the cardiac action potential.

There are five main classes in the 'Vaughan-Williams' classification.

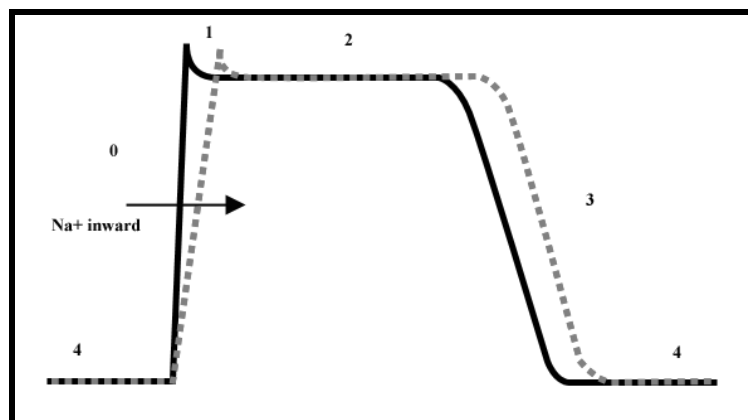
9.1.1 Class I agents

These anti-arrhythmic agents interfere with the functioning of the Na^+ -channel. Based on its effect on the cardiac action potential the class I agents are subdivided in Class IA, IB and IC.

9.1.1.1 Class IA agents

The main property of a Class IA agent is blocking the fast Na^+ -channel. This effect results in a depression of the phase 0 depolarisation and a reduction of the maximum upstroke velocity of the action potential (V_{max}) (Figure 13). The other action mechanisms include a decrease in conductivity and therefore an increase in the refractory period.

Figure 13. A class IA anti-arrhythmic drug reduces V_{max} during phase 0 thereby increasing the duration of the action potential.

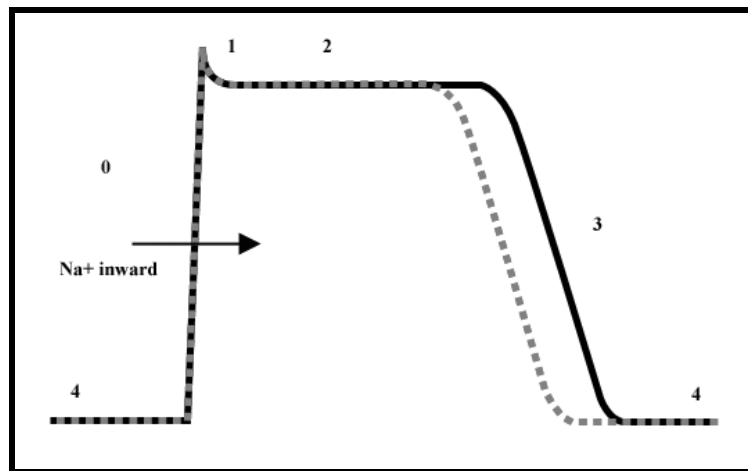


The most important Class IA agents are quinidine, procainamide and disopyramide.

9.1.1.2 Class IB

Class IB anti-arrhythmic drugs also block Na^+ -channels but have little or no effect at slow heart rates because of their fast onset and offset kinetics. The effects are more pronounced at faster heart rates such as ventricular tachycardia. These drugs reduce electrical conductance among the cells by stabilizing the inactive state of Na^+ -channels resulting in a slower heart rate. The duration of the action potential and the refractory period are reduced but there is no effect on the V_{max} (Figure 14).

Figure 14. A class IB anti-arrhythmic drug shortens the action potential duration.

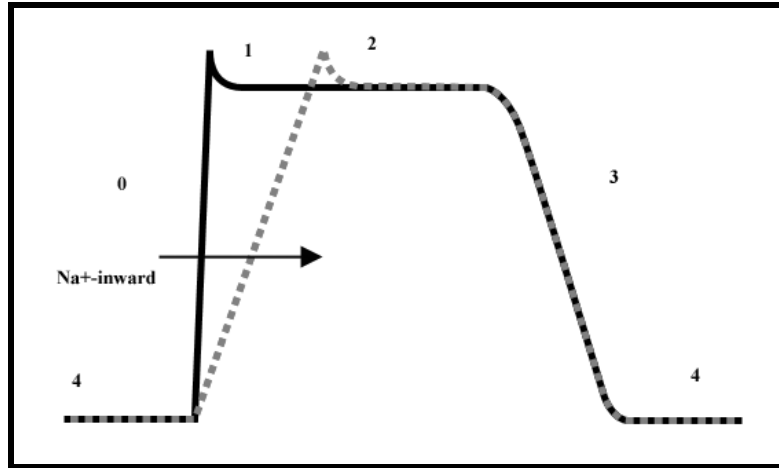


Important class IB agents include lidocaine, mexiletine, tocainide and phenytoin.

9.1.1.3 Class IC agents

Class IC anti-arrhythmic drugs strongly depress the V_{max} and thus phase 0. A decrease in conductivity and excitability of the cardiac cells but minimal effects on the duration of the action potential is observed (Figure 15). Class IC agents have the most potent sodium channel blocking effects.

Figure 15. Class IC anti-arrhythmic drugs cause a major decrease in V_{max} .



Class IC agents are propafenone, flecainide, cibenzoline, encainide and moricizine.

9.1.2 Class II agents

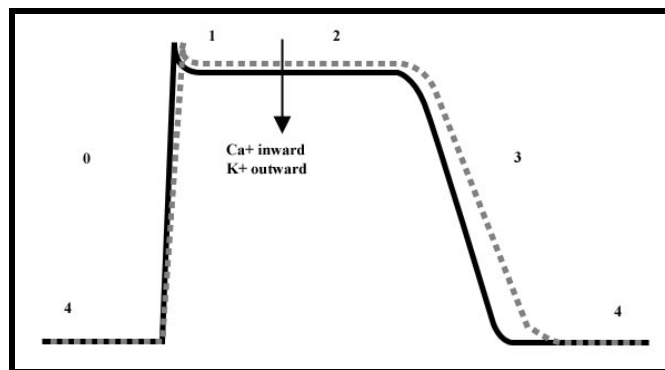
Class II anti-arrhythmic drugs are classified as beta receptor blockers. Beta receptors can be separated into those that affect predominantly the heart (β_1) or the bronchi and those that affect blood vessels (β_2). In low doses, Class II anti-antiarrhythmic drugs decrease the sympathetic activity on the heart by selectively blocking the effect of catecholamines and β_1 -adrenergic receptors. In high doses, the selective β_1 blockers also block β_2 receptors.

Frequently used class II anti-arrhythmic drugs include propranolol, esmolol and metoprolol.

9.1.3 Class III agents

The class III anti-arrhythmic drugs predominantly block the K^+ -channels resulting in a prolongation of the action potential. These agents have less effect on the Na^+ -channels, therefore conduction velocity is not changed (Figure 16).

Figure 16. Class III anti-arrhythmic drugs increase the cardiac action potential duration by blocking the K⁺ channels.



By maintaining the normal conduction velocity and increasing the refractory period, class III anti-arrhythmic drugs may prevent or terminate re-entry arrhythmias.

Class III agents include amiodarone, azimilide, bretylium, dofetilide, tedisamil, ibutilide, sematilide and sotalol.

9.1.4 Class IV agents

These agents have a blocking effect on the Ca²⁺-channels which control calcium influx into vascular smooth muscle cells and myocytes. As such they shorten phase 2 of the cardiac action potential and play a major role in the excitation-contraction cycle, the sino-atrial pacemaker activity and AV conduction. All these interactions lead to a reduction in heart rate.

Class IV drugs are verapamil and diltiazem.

9.1.5 Class V agents

Because some important anti-arrhythmic drugs could not be classified based on their main property in the previous described classes, a new group has been developed in the 'Vaughan-Williams' classification. This class includes digoxin and adenosine.

Digoxin inhibits the Na⁺/K⁺ pump which lead to increased Na⁺ levels. This in turn slows down the extrusion of Ca²⁺ via the Na⁺/Ca²⁺ pump. This effect causes an increase in the length of phase 4 and phase 0 of the ventricular action potential. This effect together with an increase of the vagal activity via its central action on the central nervous system leads to a decrease of the ventricular rate.

Adenosine enhances the K^+ efflux and inhibits the Ca^{2+} influx. It produces AV block and an indirect effect on the refractory period of atrial tissue.

Human data

In human medicine class IC is the first choice anti-arrhythmic drug for atrial fibrillation whereby a success rate of 90% has been described. However, success rate decreases with the duration of AF. Also class III seems to be very effective in the treatment of atrial fibrillation and flutter. In contrast class II and IV are described as being less valuable in atrial fibrillation. Class IV drugs are more used to control ventricular frequency than for conversion to sinus rhythm (Crijns et al., 1999; Kerin et al., 1996; Van Gelder et al., 1999).

Equine data

Quinidine sulphate (QS), a class IA drug, is the “gold” standard treatment of atrial fibrillation in horses. However, the duration of AF and the presence of underlying cardiac disease do have an important effect on success rate. For recent-onset AF (less than 2 weeks), quinidine gluconate has been recommended whereby boluses of 1-2.2 mg/kg are administered intravenously every 10 minutes until effect or until a maximum of 12 mg/kg is reached. However, compared to the oral administration, the intravenous administration of quinidine has been associated with a higher risk for toxic side-effects. Oral QS medication is the pharmacological treatment of choice for AF in horses: QS is administered at 22 mg/kg via nasogastric intubation every 2 hours until conversion to sinus rhythm or observation of side effects. The therapeutic plasma concentration is 2 to 5 $\mu\text{g/ml}$. If plasma concentration is more than 4.0 $\mu\text{g/ml}$, treatment intervals should be prolonged up to every 6 hours (Reef, 1999).

QS treatment has an efficacy of 85% in horses with an AF duration of less than 2 months and without detectable cardiac disease (Deem and Fregin, 1982; Reef et al., 1988). Nevertheless, in up to 76% of treated horses, side effects including urticaria, nasal oedema, colic, diarrhoea, laminitis, anaphylactic shock, hypotension, decreased cardiac contractility, increase in QRS duration, tachycardia, ventricular arrhythmias, syncope or sudden death may appear (Reef et al., 1988, 1995). When significant side effects occur, QS administration should be discontinued. Hypotension can be treated with phenylephrine (0.1-0.2 $\mu\text{g/kg/min}$ to effect)

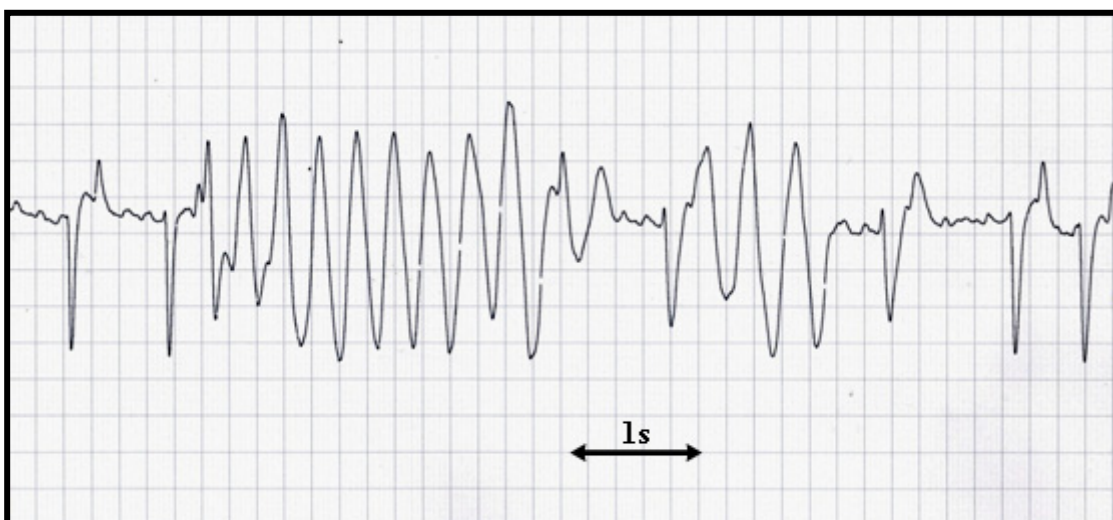
while sustained supraventricular or ventricular tachycardia (< 100 beats/min) is generally treated with digoxin (0.0022 mg/kg) but one must be aware that digoxin increases quinidine plasma levels. When toxic quinidine signs are observed, intravenous sodium bicarbonate should be administered to bind free quinidine.

Intravenous propranolol (0.03 mg/kg), lignocaine ($20-50$ μ g/kg/min) or magnesium sulphate ($1-2.5$ g/450 kg/min) can also be used in horses with quinidine-induced tachycardia to control heart rate (Reef, 1999).

Despite the high success rate, quinidine's toxicity and the fact that in several countries the drug becomes difficult to obtain have stimulated research into alternative therapies.

Intravenous cibenzoline (0.1 mg/kg/min), a class IC drug (with additional class III and IV properties), was unsuccessful in a horse with AF and was associated with severe ventricular pro-arrhythmia (van Loon, 2003). Intravenous flecainide (2 mg/kg at 0.2 mg/kg/min), a class IC drug, was reported to be effective in horses with acute, experimental AF (Ohmura et al., 2000) but failed to convert horses with naturally-occurring chronic AF. Horses from the latter group even developed potentially dangerous ventricular dysrhythmias (Figure 17) (van Loon et al., 2004).

Figure 17. Continuous ECG recording from a horse showing ventricular dysrhythmia during flecainide treatment.



Sotalol, a class III drug, has been used in three horses with AF (0.75-1.25 mg/kg IV infusion over 15 minutes) and was not associated with side-effects. However, sinus rhythm could not be restored (van Loon, 2003).

Recently, the use of diltiazem (0.125-1.125 mg/kg over 2 minutes), a class IV drug, was investigated in healthy horses. The drug induced a dose-dependent suppression of the sinoatrial node and a decrease in atrial blood pressure, resulting in a slower ventricular response to experimentally applied atrial stimuli. The authors of this study hypothesized that the drug might be used in horses that show quinidine-induced supraventricular tachycardia (Schwarzwald et al., 2005, 2007a) but so far no data are available.

9.2 Non-pharmacological approaches to AF

In human medicine, the mainstay of managing AF is drug therapy. However, when drug therapy is ineffective or not tolerated, a non-pharmacological approach is available whereby general anaesthesia is usually required (Scheinman and Morady, 2001).

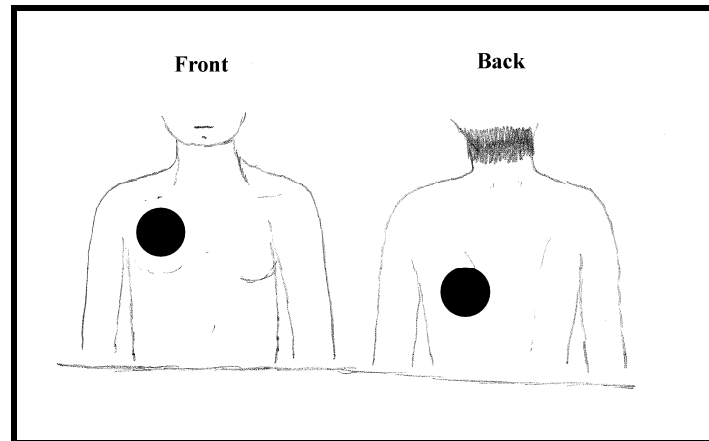
9.2.1 External electrical cardioversion

Human data

Electrical cardioversion is used as treatment of cardiac arrhythmias such as AF, ventricular tachycardia, and ventricular fibrillation. The purpose of the direct current shock delivery is to depolarize the whole myocardium, bringing it into a refractory state, thereby interrupting re-entry circuits and thus terminating the arrhythmia. The term ‘defibrillation’ is used for shock delivery during ventricular fibrillation. The term ‘cardioversion’ is used for shock delivery during atrial fibrillation or ventricular tachycardia. Since some kind of ventricular rhythm is still present during cardioversion, shock delivery should be synchronized with the R wave of the QRS complex. Indeed shock delivery onto a T wave could induce ventricular fibrillation.

During external cardioversion the electrical shock is delivered through the chest using two paddles or electrodes placed on the skin. One paddle is positioned at the right side of the sternum at the level of the first or the second rib while the other paddle is placed at the infraclavicular region at the left side of the vertebrae (Figure 18) (Botto et al., 1999).

Figure 18. Paddle placement for external electrical cardioversion. One paddle is positioned at the right side of the sternum at the level of the first or the second rib and the other paddle is placed at the infra-clavicular region at the left side of the vertebrae.



Success rate of external electrical cardioversion for chronic atrial fibrillation ranges from 70-90% but depends mainly on the transthoracic impedance. As transthoracic impedance is correlated with body weight, cardioversion success rate is lower in obese patients (Lévy et al., 1992). In these patients internal cardioversion is recommended.

Although the success rate is high, recurrence of AF within 2 weeks after cardioversion is a common problem (Tieleman et al., 1998). Therefore, a pre-treatment with an anti-arrhythmic drug is recommended because it lowers the vulnerability of the atria. Amiodarone has been proven to be successful in preventing immediate recurrence of atrial fibrillation after successful electrical cardioversion (Capuci et al., 2000; Crijns et al., 1999; Gorenek et al., 2006).

Equine data

Frye et al. (2002) attempted to use external electrical cardioversion in 2 horses with AF. Several attempts were unsuccessful and, finally, only the smallest horse (393 kg), with the shortest AF duration (3 weeks), could be converted with a 200 J shock, but this only after pre-treatment with quinidine sulphate.

9.2.2 Internal electrical cardioversion

Human data

Besides external cardioversion, human patients can be treated with internal, usually transvenous, electrical cardioversion. During this procedure, one cardioversion catheter is placed high in the right atrium (RA) against the atrial wall and a second is positioned in the

coronary sinus (CS) or the left pulmonary artery, which is in close contact with the left atrium. Low energy (up to 30 J), R wave synchronized shocks, with one catheter in the RA and one catheter in the CS, result in restoration of sinus rhythm in the majority of patients.

Similar to the external approach, early recurrences of AF (Duytschaever et al., 2000, 2002; Lévy et al, 1997; Ricard et al., 2003; Timmermans et al., 1998; Van Noord et al., 2002) can be minimized by pre-treatment with anti-arrhythmic drugs such as amiodarone.

Equine data

Successful internal cardioversion under general anaesthesia has been described in horses (McGurrin et al., 2003, 2005; van Loon, 2001). Placement of the catheters, into the right atrium and left pulmonary artery, was performed in the standing, sedated animal. Mean cardioversion energy was 162.2 \pm 10.2 joule. Despite the importance of early recurrence of AF in human patients, this problem has not been reported in horses yet. This might be explained by the fact that during conventional QS treatment, the therapeutic drug level at the time of conversion prevents AF to reoccur.

9.2.3 Ablation of AF

Human data

Ablation is defined as the removal of material of the surface of an object by vaporization, chipping or other erosive processes. Ablation therapy using radiofrequency waves in the heart is used to cure cardiac arrhythmias. In this context, the term ablation is often used as laser ablation, a process by which the molecular bonds of a material are dissolved by a laser.

In human patients catheter ablation is used for both rate and rhythm control of AF. The procedure can usually be performed using sedatives and local anaesthetics.

9.2.4 Atrioventricular junction ablation

With this procedure, an ablation catheter is transvenously inserted and positioned at the atrioventricular node using fluoroscopy. Using radiofrequency energy, the atrioventricular

junction is destroyed resulting in atrioventricular block. Consequently, a permanent pacemaker is required to maintain a normal ventricular function. This procedure does not terminate atrial fibrillation, but is a form of rate control. This procedure is used in symptomatic patients with AF who are refractory to treatment with several anti-arrhythmic drugs and have an uncontrolled ventricular rate (Scheinman and Morady 2001).

9.2.5 Linear catheter ablation

This technique has been developed based upon the re-entry theory and attempts to decrease the number of wavelets by decreasing the amount of continuous, vulnerable atrial tissue. Linear interruptions are made in the right or left atrial myocardium (Scheinman and Morady, 2001). However, success rate only ranges from 0 to 58% (Ernst et al., 1999; Haissaguerre et al., 1996; Pappone et al. 1999).

9.2.6 Focal catheter ablation

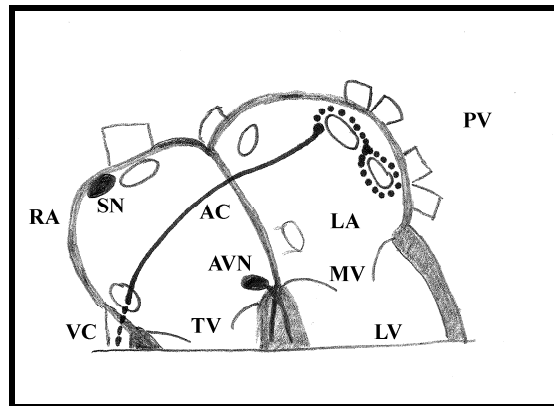
In a similar way multiple electrode catheters can be inserted in the atria to register the activation patterns. These are used to check for ectopic foci in the atria that are responsible for initiation of AF. Once an ectopic focus is determined, this site is ablated to prevent further discharge and thus AF. In 95% of the cases the focus is located within the pulmonary vein but other sites include right atrium, left atrium, coronary sinus, superior vena cava or vein of Marshall (Shah et al., 1999). Success rate ranges between 62 and 86% and in 7 to 75% of the cases a second or third attempt of ablation is required (Chen et al., 1999; Haissaguerre et al., 1998) because of the presence of multiple foci (Scheinman and Morady, 2001).

9.2.7 Segmental or total pulmonary vein ablation

Ectopic foci are located in the pulmonary veins in 95% of the patients, therefore total or partial ablation of the ostia of the pulmonary veins is performed (Figure 19). This ablation around the entrance site of the pulmonary vein into the left atrium prevents the electrical pulses, coming from an ectopic focus in the pulmonary vein, to be transferred to the atrial

myocardium. Success rate ranges from 67 and 90% for patients with paroxysmal AF and 60 to 67% for patients with chronic AF (Natale et al., 2000; Lau et al., 1999).

Figure 19. Illustration of pulmonary vein ablation.



RA: right atrium. VC: vena cava. SN: sinus node. AVN: atrio-ventricular node. LA: left atrium. LV: left ventricle. PV: pulmonary veins; AC: ablation catheter. TV: tricuspid valves. MV: mitral valves.

Equine data

Ablation requires very precise positioning of a catheter in the heart. Due to technical limitation and the large equine thorax, fluoroscopy does seldom allow such accurate positioning. Overlying lung tissue and the fact that apical echocardiographic views cannot be obtained in mature horses, hamper an ultrasound-guided approach. In addition, for the same reasons, an ectopic focus cannot be localised in horses. Therefore, catheter ablation cannot be performed in horses with AF, at present.

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SCIENTIFIC AIMS

Despite the importance of atrial fibrillation (AF) in horses and extensive research about electrical and contractile remodeling in goats and dogs, little is known about AF pathophysiology in horses. Indeed, when horses are referred to clinic, the exact duration of AF is usually unknown. In addition, the presence of predisposing factors cannot fully be ruled out by the clinical exam, serum biochemistry and cardiac ultrasound. As such, studying AF pathophysiology in horses with natural AF development is difficult because many unknown factors might be present. These inconveniences can be overcome by studying healthy horses in which AF is experimentally induced and maintained. As such, an exact AF duration can be chosen, and baseline values can be compared with values during maintained AF and after restoration of sinus rhythm. Acute AF-associated remodeling provides information about the acute phase of AF and could be applicable to paroxysmal AF. More long-term remodeling is important to understand alterations that occur with chronic AF and especially to investigate the reverse remodeling process after restoration of sinus rhythm. This information is important, not only for diagnosis and treatment, but especially for prognosis and aftercare after cardioversion.

Therefore, the first aim of our study (Section 1) was to study the electrical and contractile remodeling associated with AF in healthy horses, as a result of both an acute (Chapter 1) and a chronic (Chapter 2) AF episode.

Because 'lone' AF horses have a good prognosis after conversion of AF allowing them to return to full competition, rhythm control is indicated in most horses. Nevertheless, the most commonly used drug for AF treatment in horses, quinidine sulphate, is becoming difficult to obtain in many countries. In addition, toxic side effects are commonly encountered during quinidine treatment. In human medicine, quinidine has almost completely disappeared as pharmacological treatment. Many other less toxic but still effective drugs for rhythm control

are nowadays available. In addition, especially in drug refractory AF human patients, electrical cardioversion of AF has become a mainstay in the treatment of AF.

As such, the second aim of our study was to investigate new pharmacological (Section 2) and non-pharmacological (Section 3) approaches for the treatment of AF in horses. Drugs used in human medicine, such as propafenone (Chapter 3) and amiodarone (Chapter 4, 5 and 6) were evaluated as pharmacological treatment of AF in horses. Finally, custom-made cardioversion catheters were developed to achieve transvenous electrical cardioversion of AF in horses (Chapter 7).

SECTION 1

REMODELING AND REVERSE REMODELING IN HORSES ASSOCIATED WITH ATRIAL FIBRILLATION

CHAPTER 1

REMODELING AND REVERSE REMODELING ASSOCIATED WITH SHORT-TERM ATRIAL FIBRILLATION

ATRIAL AND VENTRICULAR ELECTRICAL AND CONTRACTILE REMODELING AND REVERSE REMODELING DUE TO SHORT-TERM PACING-INDUCED ATRIAL FIBRILLATION IN HORSES

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Summary

In human medicine, paroxysmal atrial fibrillation (AF) induces electrical, contractile and structural remodeling leading to AF stabilisation. Little is known about atrial remodeling during AF in horses. The hypothesis was that induced AF produces rapid atrial electrical and contractile remodeling in horses.

In six horses a pacemaker and a neurostimulator were implanted. With the latter burst pacing was used to induce AF for 7 days, the pacemaker was used to study atrial and ventricular electrophysiology (atrial fibrillation cycle length [AFCL], AF duration and atrial and ventricular effective refractory period [AERP and VERP] at different pacing cycle lengths [PCL]). Contractile remodeling of atrium and ventricle were assessed with calculation of fractional shortening (FS). Measurements were performed at baseline, a 7-day AF-period and a 2-day recovery period.

Within 4 and 12 hours, AF resulted in atrial electrical and contractile remodelling. During the AF period a progressive shortening of the AERP ($261\pm 39\text{ms}$ to $171\pm 18\text{ms}$ at a PCL of 1000ms , $p<0.0001$), an attenuation of the AERP rate adaptation and a decrease in AFCL ($239\pm 39\text{ms}$ to $194\pm 7\text{ms}$, $p<0.0001$) occurred. AF duration increased progressively and became persistent in two animals. VERP did not change significantly. AF resulted in a progressive decrease in atrial FS occurred ($12\pm 3\%$ to $0\pm 2\%$, $p<0.05$). Upon restoration of sinus rhythm, values returned to baseline within 48 hours.

Atrial electrical and contractile remodeling appears rapidly. Reverse remodeling occurred within 2 days. These observations suggest that early conversion of AF might be beneficial for success rate and early returning to training.

Introduction

In human patients the natural history of atrial fibrillation (AF) is characterized by an evolution from short self terminating episodes of AF (paroxysmal AF) to longstanding episodes of AF requiring pharmacological or electrical cardioversion (persistent AF). If no attempts to restore sinus rhythm are made, AF becomes permanent (Fuster et al., 2006). Epidemiological data suggest that 29% of the patients with paroxysmal AF without underlying heart disease progressed to permanent AF within 15-30 years (Jahangir et al., 2007). This evolution from paroxysmal to permanent AF is caused by electrical, contractile and structural changes and their interaction (Allessie et al., 2002; Morillo et al., 1995; Schotten and Allessie, 2001; Schotten et al., 2003; van Loon et al., 2001a; 2001b; Wijffels et al. 1995). Human data and experimental work in dogs, goats and ponies, has shown that AF results in shortening of the atrial effective refractory period (AERP), attenuation of the AERP rate adaptation and reduction of the atrial contractile function (Morillo et al., 1995; van Loon et al., 2001; Wijffels et al., 1997). Schwarzwald and co-workers (2007), described that contractile remodeling also occurs in horses with chronic naturally-occurring AF. van Loon et al. (2001) described that, after 6 months of experimentally induced AF in ponies, electrical and contractile reverse remodeling occurred after 10 days and 1-2 months, respectively.

In human patients and in dog and goat models of AF, structural changes such as alterations in gap-junctions, myolysis, disruption of the sarcoplasmic reticulum, accumulation of glycogen or evidence of apoptosis and fibrosis, might occur if AF exists for more than 5 weeks (Ausman et al., 1997; Elvan et al., 1997; Li et al., 1999). Light and electron microscopic results of transvenously taken atrial biopsies of ponies with 6 months of experimentally maintained AF revealed no structural changes such as myolysis (van Loon et al., 2002a).

In a hospitalized population the prevalence of AF with unknown duration approaches 2.5% (Else and Holmes, 1971). The prevalence of paroxysmal AF in finishing racehorses is 0.29% while it is 1.39% in slow finishing and non-finishing racehorses (Ohmura et al., 2003). Whether repeated bouts of paroxysmal AF in horses promote persistence of the arrhythmia by atrial remodeling is yet unknown. Although it has been suggested that AF duration influences cardioversion success rate and quinidine associated side effects (Reef et al., 1988; Reef et al., 1995), little is known about AF remodeling and its time-course.

We hypothesize that, in horses, AF results in rapid electrical and contractile remodeling. In horses with naturally-occurring AF the exact duration of the arrhythmia is generally unknown and distinct underlying pathology cannot be ruled out. Therefore, in the current study, an experimental AF model in healthy horses was used to allow baseline measurements, to know exact AF duration, to make repeated measurements during the AF period and to rule out underlying disease. As such, the observed alterations were attributable to AF.

Material and Methods

The study was performed at the University of Ghent (Belgium) and was supported by the Special Research Fund, Ghent University (Belgium). This research was approved by the Ethics Committee of the Faculty Veterinary Medicine, Ghent University (Belgium).

Animals and study protocol

Six healthy trotter horses with a mean \pm SD age of 4.0 \pm 0.8 years, a mean weight of 473 \pm 32 kg and a mean height at the withers of 157 \pm 2 cm, were studied. A clinical examination, a complete blood analysis, electrocardiography and echocardiography including two-dimensional, M-mode and Colour Flow Doppler were performed to exclude animals with underlying heart disease.

In all horses, a dual chamber pacemaker (Thera D(R), Medtronic), a neurostimulator (SolettraTM 7426, Medtronic) and leads (Fineline[®] II, Guidant) were implanted as previously described by van Loon et al. (2002b). With the neurostimulator, intermittent burst pacing (1 second; 3 times threshold, 20 Hz, every 2 seconds) was applied to induce bouts of AF. The dual chamber pacemaker was used to study atrial and ventricular electrophysiology.

The study consisted of 3 study periods: a baseline period, a 7-day AF-period and a 2-day recovery-period. In each period repeated electrophysiological and echocardiographic measurements were performed in the unsedated horse within 20 minutes after spontaneous restoration of sinus rhythm. For the latter, under continuous telemetric ECG monitoring, the neurostimulator was temporarily switched off to allow sinus rhythm to restore spontaneously. After these measurements, the pulse generator was immediately switched on for further maintenance of AF.

At the end of the 7-day AF-period, the pulse generator was permanently switched off to allow permanent restoration of sinus rhythm. If spontaneous conversion to sinus rhythm did not occur within 3 days, flecainide (Tambocor[®], 150 mg/15mL, Meda Pharma) was administered at 0.2 mg/kg/min up to a maximum total dose of 2 mg/kg (Ohmura et al., 2000). Before, during and up to 6 hours after drug administration, clinical status, intra-atrial and surface electrocardiograms were monitored. Prior to the pharmacological treatment, haematological and biochemical blood analysis was performed and electrolyte concentrations were determined.

After the administration of intravenous flecainide, all data collected within the first 6 hours, which is the elimination half-life of flecainide, were excluded from the study to avoid pharmacological interference.

Electrophysiological measurements

At baseline, during the AF-period and during the recovery-period, atrial effective refractory period (AERP) and ventricular effective refractory period (VERP) were measured at three times threshold amplitude. During pacing with a fixed pacing interval (S1-S1) an extra stimulus (S2) was introduced with a coupling interval (S1-S2) below the expected refractory period. The coupling interval was then increased in steps of 8 ms until capture of the extra stimulus occurred (the atrial or ventricular S2 was followed by a P wave or QRS complex on the surface ECG, respectively). The longest S1-S2 interval without capture was taken as the effective refractory period. AERP was measured at pacing cycle lengths (PCL) of 1000ms (60 beats/min), 800ms (75 beats/min), 500ms (120 beats/min) and 333ms (180 beats/min). VERP was measured at PCL of 1000ms, 800ms and 500ms. A 2-minute adaptation was allowed at each pacing cycle length. AERP and VERP were always determined 3 times to obtain a mean value. The heart rate was measured during AF and after restoration of sinus rhythm.

AF duration was measured as the time between disabling the neurostimulator and spontaneous conversion to sinus rhythm. The AFCL was measured as an average time between two successive atrial depolarisation waves during a 10s atrial intracardiac electrogram obtained from the pacemaker (Morillo et al., 1995).

Time points of all measurements, before, during the AF-period and during the recovery period are illustrated on Figures 3-6.

Echocardiographic views and measurements

Echocardiographic views were obtained using standardised imaging techniques with a 2.5 MHz sector transducer with a maximal depth of penetration of 30 cm (GE Vingmed CFM 800 SV). A single-lead electrocardiogram was recorded simultaneously. Recordings were stored on digital MO-disks and on video tape for retrospective analysis.

Every echocardiographic variable was determined from 5 different cardiac cycles to obtain a mean value. All echocardiographic measurements were performed on 5 different days at baseline and after 12, 24, 36, 48, 60, 72, 96 and 168 hours of maintained AF (both during AF and during sinus rhythm) and after 12, 24, 36, and 48 hours after termination of the AF-period. Cycles during and immediately after a second-degree atrioventricular block or a spontaneous atrial premature beat were excluded from analysis. During AF, cardiac cycles with an RR interval between 1333 to 1090 ms (45 to 55 beats/min) were selected.

Left atrial (LA) internal diameters (LAD) and LA cross-sectional area (LAA) were measured from a right parasternal long-axis view. LAD was measured from the interatrial septum, close to the mitral annulus to the atrial free wall (Figure 1). For every cardiac cycle 4 time points were determined to perform these measurements: point “p” was at the onset of the P wave; point “a” was during maximal atrial contraction, point “d” was at the end of the ventricular diastole and point “s” was at the end of ventricular systole. Due to the absence of an atrial contraction, point “p” and “a” could not be determined during AF.

Left atrial fractional shortening (LA-FS) was calculated as suggested by Piotrowski et al. (2000) with the following formula:

$$\text{LA - FS\%} = \frac{\text{LADp} - \text{LADa}}{\text{LADp}} \times 100$$

Left atrial fractional area change (LA-FAC) was calculated as suggested by Piotrowski et al. (2000) with the following formula:

$$\text{LA - FAC\%} = \frac{\text{LAAp} - \text{LAAa}}{\text{LAAp}} \times 100$$

On the right parasternal short-axis view of the LA and aorta, internal diameters of the LA and the aorta were obtained (Figure 2). For the internal short-axis diameter of the LA (LA_{sx}) callipers were placed in a line extending from and parallel to the commissure between the noncoronary and left coronary aortic valve cusps to the distant margin of the left atrium. For the internal short-axis diameter of the aorta at valvular level (AODV), callipers were placed along the commissure between the noncoronary and right coronary aortic valve cusps. For each time point (p, a, d, s) during the cardiac cycle the ratio $\text{LA}_{\text{sx}}/\text{AODV}$ was calculated (Young, 2004).

On a standard M-mode of the ventricles the left ventricular internal diameter, the interventricular septal thickness and the left ventricular free wall thickness were measured in diastole and systole and the left ventricular FS was calculated.

Colour Flow Doppler examinations of the mitral, tricuspid, aortic and pulmonic valves were performed.

Statistical analysis

Data are shown as mean \pm standard deviation. All analyses were based on the mixed model with horse as random effect and time as categorical fixed effect. All time points were compared with the baseline value at time zero, using Dunnett's method to adjust for multiple comparisons and a global significance level of 5%.

Figure 1. On the right parasternal long-axis view, latero-lateral left atrial diameter (LAD) was measured from the lateral free wall to the interatrial septum. The circular line represents the left atrial cross-sectional area (LAA). RA: right atrium. RV: right ventricle. TV: tricuspid valve. LA: left atrium. LV: left ventricle. MV: mitral valve. IVS: interventricular septum

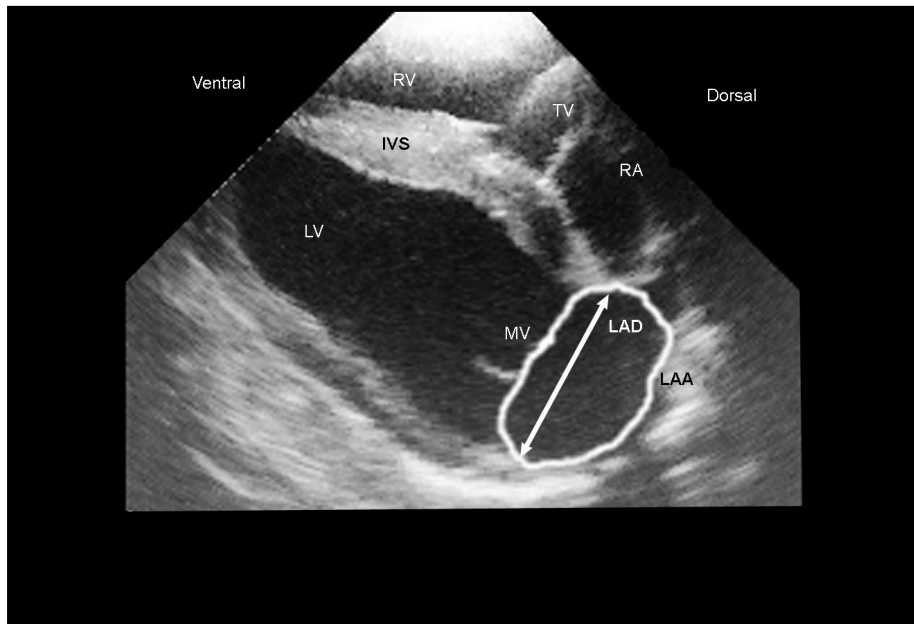
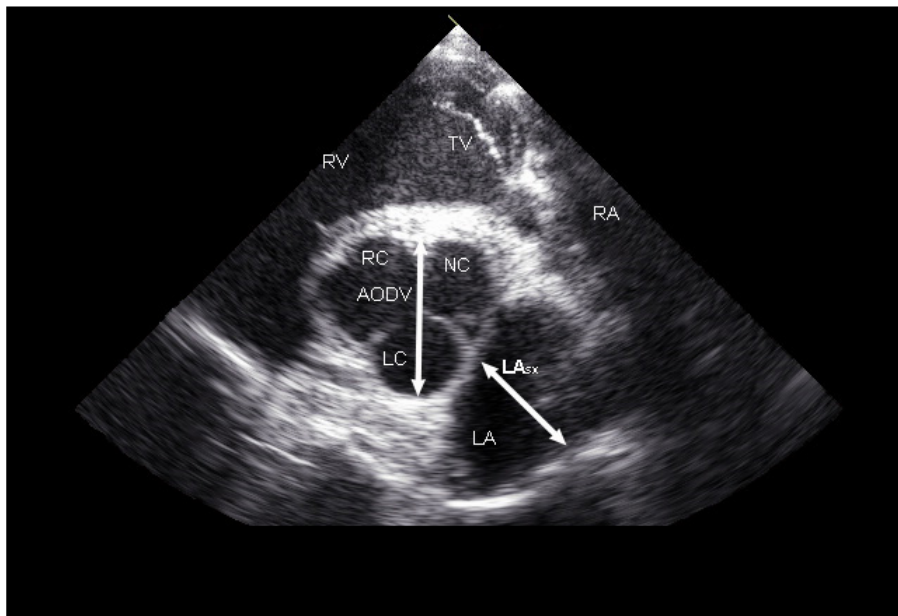


Figure 2. A right parasternal short-axis view of the left atrium and aorta. The diameter of the left atrium (LASx) and aorta (AODV) were obtained. To measure the internal short-axis diameter of the aorta calipers were placed along the commissure between the noncoronary and right coronary aortic valve cusps. To obtain the internal short-axis diameter of the left atrium (LASx), calipers were placed in a line extending from and parallel to the commissure between the noncoronary and left coronary aortic valve cusps to the distant margin of the left atrium.



RA: right atrium. RV: right ventricle. TV: tricuspid valve. LA: left atrium. RC: right coronary aortic cusp. LC: left coronary aortic cusp. NC: non-coronary aortic cusp.

Results

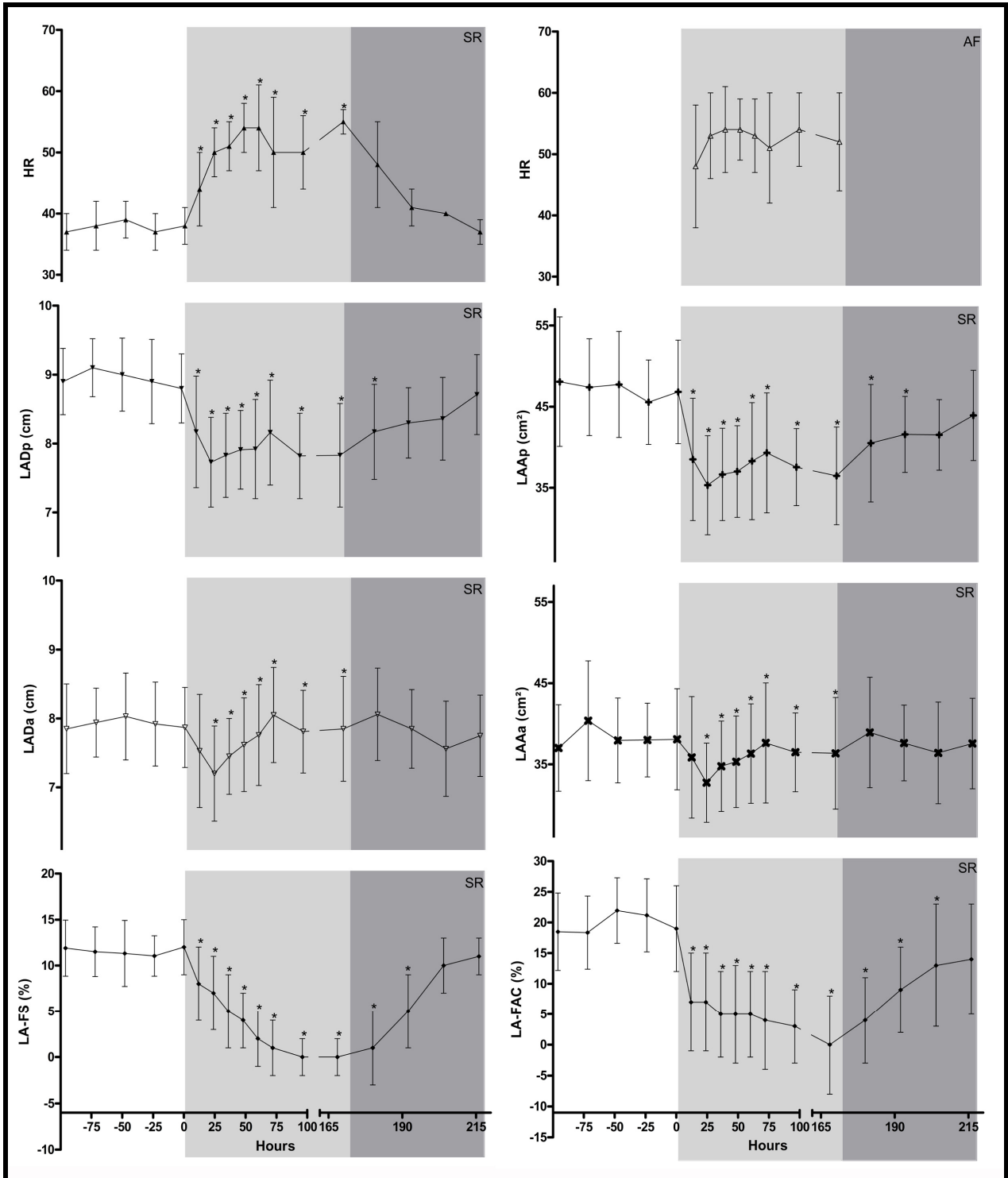
In all horses, AF could be successfully induced and maintained using the intermittent burst pacing protocol. During the AF period, when spontaneous restoration of sinus rhythm was temporarily allowed, two horses showed occasional atrial premature beats. These premature beats were no longer encountered after 4 to 5 days of recovery.

At the end of the AF period 2 horses were in persistent AF. Blood analysis and electrolyte concentrations were within normal reference ranges. In one horse sinus rhythm could be restored without any side effects after administration of 1 mg/kg flecainide intravenously. In the other horse sinus rhythm did not be restored after 2 mg/kg flecainide IV. Five minutes after termination of the flecainide infusion, without any preceding signs, this horse suddenly developed wide-QRS tachycardia, which degenerated to ventricular fibrillation and resulted in sudden death of the animal. As such, no data from the recovery period of this horse were available.

Electrophysiological measurements

In each horse, threshold for stimulation remained similar throughout the study. During the AF period a slight but significant increase in heart rate ($p < 0.01$) was observed (both during AF and during sinus rhythm) compared to baseline (Figure 3).

Figure 3. Mean (\pm SD) heart rate (beats/min), echocardiographic and contractile parameters at baseline (n=6), during the atrial fibrillation period (□) (n=6) and during the recovery period (■) (n=5) in horses. Echocardiographic parameters are measured at time points p (the onset of the atrial contraction) and a (during maximal atrial contraction).

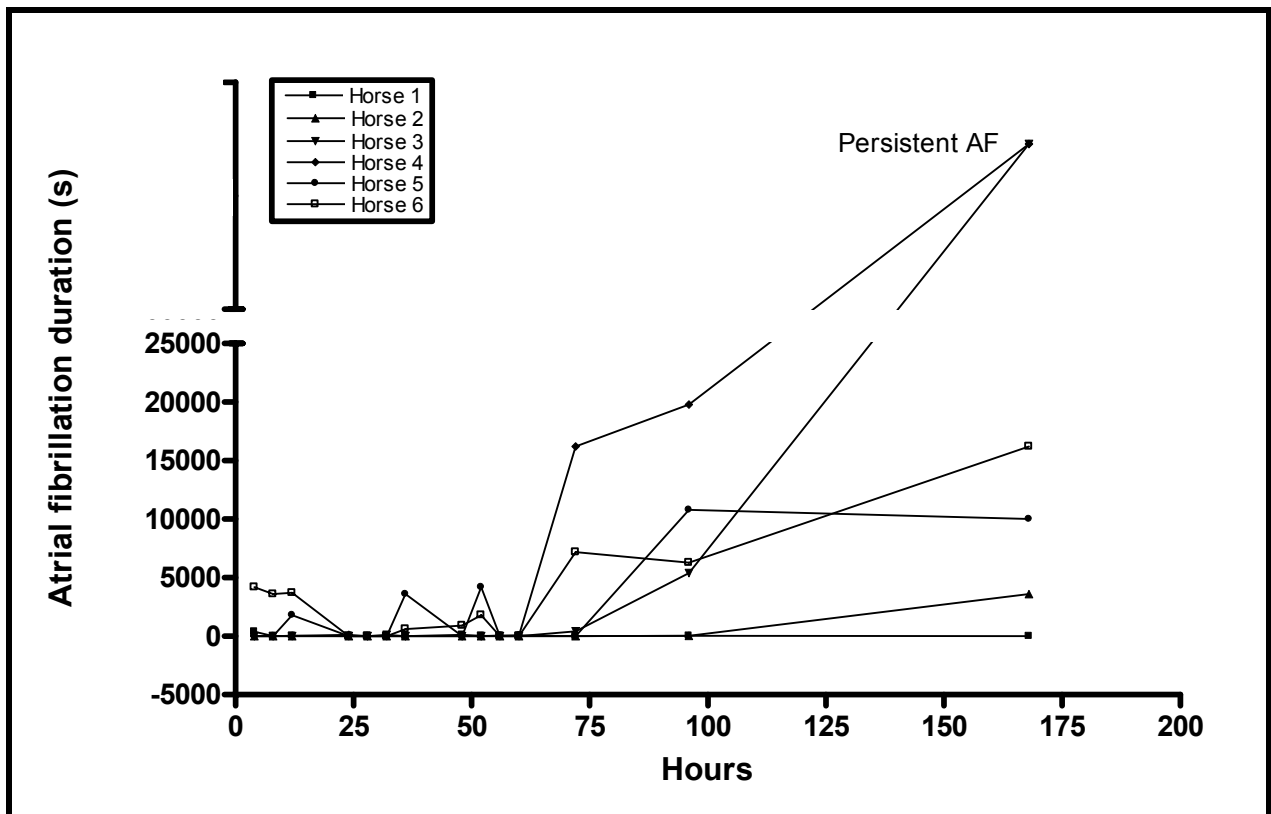


SR: measurements taken during (temporarily restored) sinus rhythm. AF: measurements taken when atrial fibrillation is present. LAD: left atrial diameter. LAA: left atrial cross-sectional area. LA-FS: left atrial fractional shortening. LA-FAC: left atrial fractional area change. * significantly different from baseline value ($p < 0.05$).

Atrial fibrillation

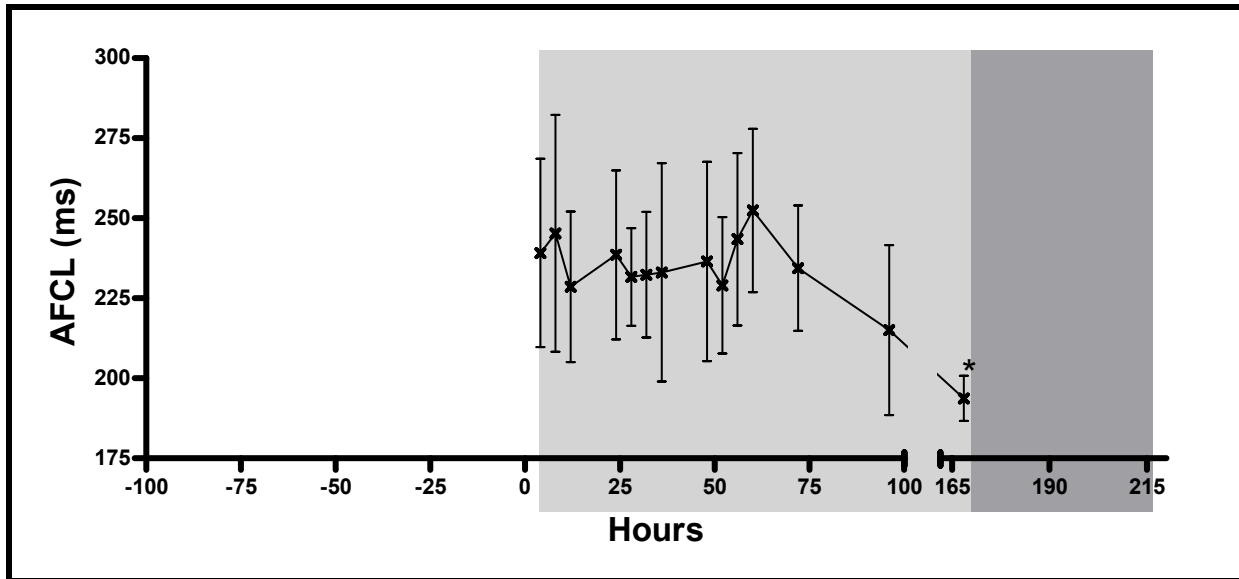
AF duration varied substantially but increased progressively during the AF period and progressed into persistent AF in two horses (Figure 4). After 4 hours of maintained AF, AFCL was 239 ± 39 ms and decreased to 194 ± 7 ms after 7 days of AF ($p < 0.0001$) (Figure 5).

Figure 4. Atrial fibrillation (AF) duration (s) of each horse (n=6) after switching off the neurostimulator during the AF period. AF duration varied substantially but increased progressively during the AF period.



s: seconds

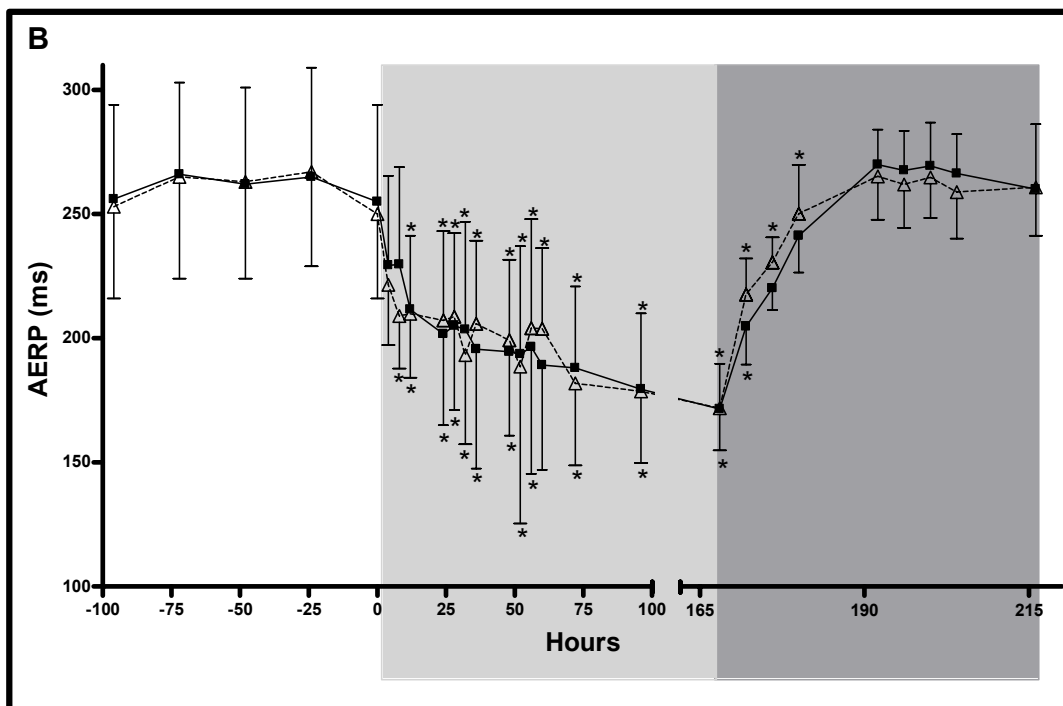
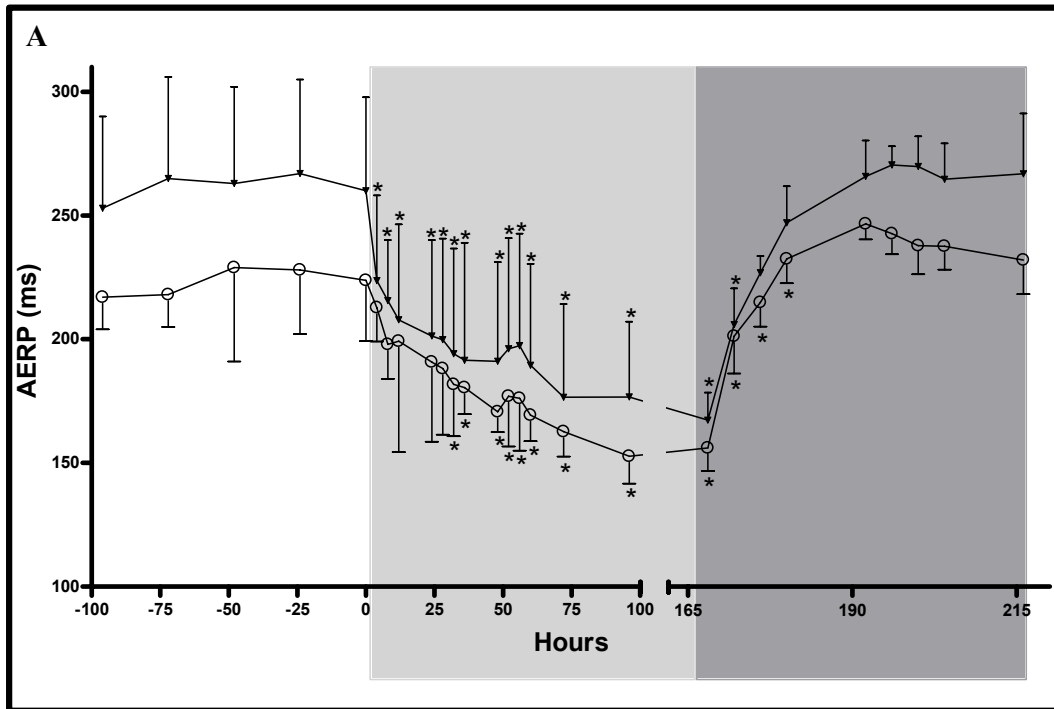
Figure 5. Mean (\pm SD) atrial fibrillation cycle length (AFCL) of horses (n=6) during the AF-period (□). (■) recovery-period. * significantly different from first measured value ($p < 0.05$).

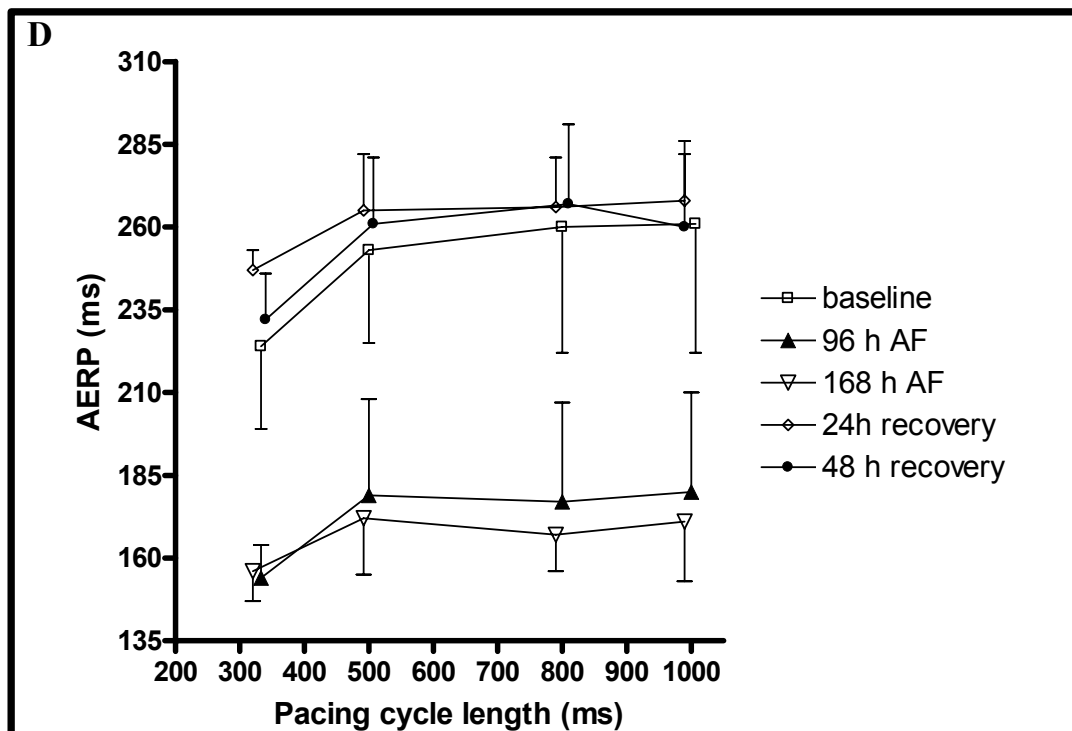
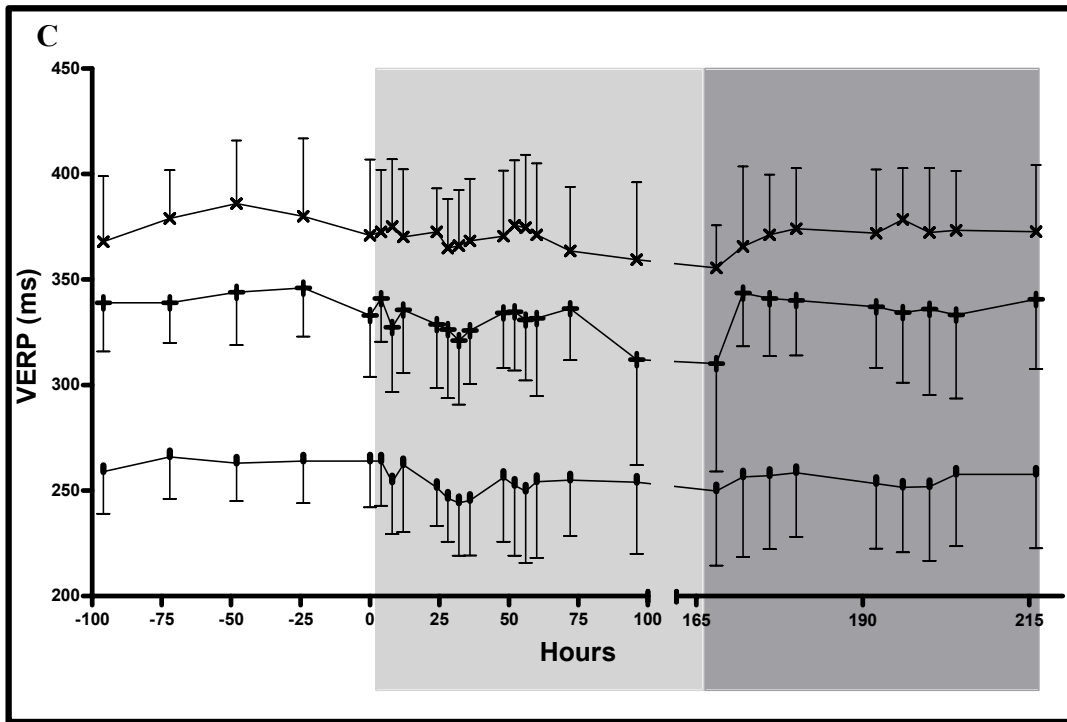


Atrial electrophysiology

Results are given in Figure 6. At baseline, AERP was 261 ± 39 ms, 260 ± 38 ms, 253 ± 28 ms and 233 ± 25 ms at a PCL of 1000, 800, 500 and 333 ms, respectively. During the maintained AF-period the AERP at a PCL of 1000, 800, 500 and 333 ms shortened significantly from 12 ($p < 0.001$), 4 ($p < 0.01$), 8 ($p < 0.01$), and 32 ($p < 0.05$) hours onwards, respectively. After seven days of maintained AF, AERP had decreased to 172 ± 18 ms, 167 ± 11 ms, 171 ± 11 ms and 156 ± 9 ms at a PCL of 1000, 800, 500 and 333 ms, respectively (Figure 6 A, B and D). A decrease in rate adaptation could be observed (Figure 6 D). Normalisation of AERP was observed within 24 hours after permanent restoration of sinus rhythm (Figure 6 A, B and D).

Figure 6. Mean (\pm SD) atrial effective refractory period (AERP) at a pacing cycle length of 333 (-O-), 800 ms (- ∇ -) (A) and 1000 (- \square -), 500 ms (- Δ -) (B) and ventricular refractory period (VERP) at a pacing cycle length of 500 (- \square -), 800 (-+), and 1000 ms (-X-) (C) at baseline (n=6), during the AF-period (\square) (n=6) and during the recovery-period (\square) (n=5) in horses. An attenuation in rate adaptation occurs as a result of atrial fibrillation (D). The first two points during the recovery period were obtained from 4 horses to avoid interference of flecainide treatment in the fifth horse.





*Significantly different from baseline value ($p < 0.05$).

Ventricular electrophysiology

The VERP was 377 ± 32 ms, 340 ± 24 ms and 263 ± 20 ms for a PCL of 1000, 800 and 500 ms, respectively. During the AF-period, a mild shortening in VERP was observed (Figure 6C). VERP measurement was never associated with repeated ventricular responses.

Echocardiographic measurements

Atrial diameters, cross-sectional, atrial FS, atrial FAC at baseline, during the AF period and during the recovery period are shown in Figure 3 and 7.

The LA-FS and LA-FAC decreased significantly within 12 to 24 hours of maintained AF ($P<0.05$). After 7 days of AF, LA-FS and LA-FAC had both decreased with 100% or more, whereby sometimes negative values were obtained (Figure 3). Within 48 hours of recovery measurements attained mean values close to the baseline value.

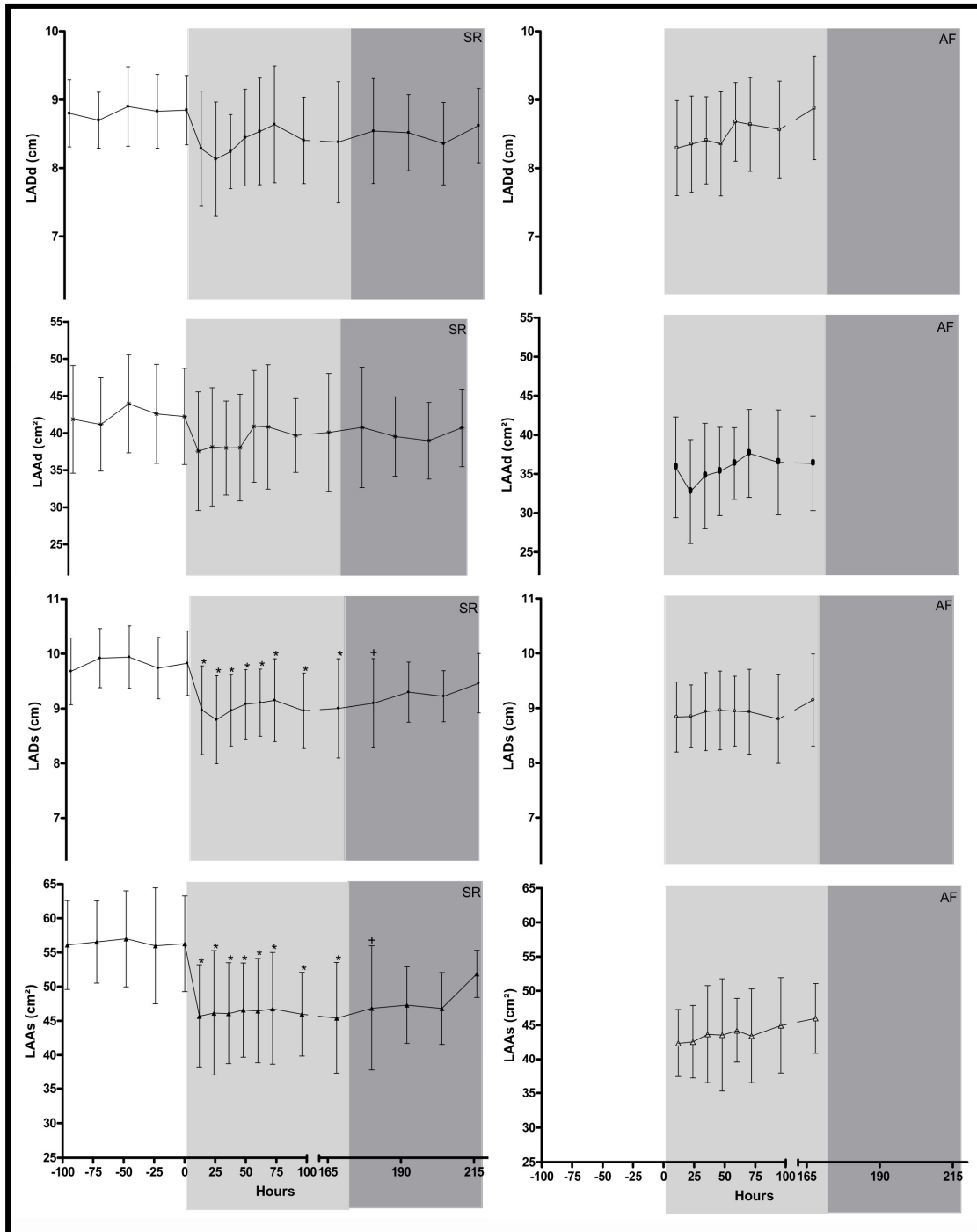
End-diastolic (d) measurements of LA diameter and AODV showed no significant differences throughout the study. A significant decrease ($P<0.01$) of all LA measurements at time point “s” was observed from 12 hours of AF onwards and normalised between 24 to 48 hours after restoration to sinus rhythm.

The ratio LA_{sx}/AOD measured at each time point during the cardiac cycle remained below 1.2 (Young, 2004).

No significant changes were detected in the interventricular septum thickness, left ventricular free wall thickness, left ventricular internal diameters and left ventricular fractional shortening.

Colour Flow Doppler of all valves revealed no differences compared to baseline.

Figure 7. Mean (\pm SD) echocardiographic parameters at baseline (n=6), during the atrial fibrillation (AF) period (■) (n=6) and during the recovery period (□) (n=5) in horses. Echocardiographic parameters are measured at time points d (at the end of ventricular diastole) and s (during maximal ventricular contraction). During the AF-period parameters are determined both in sinus rhythm (SR) and in AF (AF).



LAD: left atrial diameter. LAA: left atrial cross-sectional area. * significantly different from baseline value (p<0.05).

Discussion

The major findings of the study are as follows: (1) a short period of AF in horses (>12 hours) results in atrial electrical and contractile remodeling; (2) AFCL decreases more slowly and more time is required to develop persistent AF; (3) electrical reverse remodeling is complete within 24 hours while contractile reverse remodeling occurs more slowly.

The atrial burst pacing model of AF

The burst pacing protocol proved to be feasible and effective to induce and maintain AF in horses and was supposed to mimic naturally-occurring AF because of a similar degree in atrial contractile dysfunction (Schwarzwald et al., 2007). Besides measurements obtained during AF, the spontaneous restoration of sinus rhythm after induction of AF allows making both electrophysiological and echocardiographic measurements during normal sinus rhythm. In addition, all measurements can be performed in the unsedated horse so any drug interference can be avoided. Furthermore, in contrast to humans, dogs and goats, atrial fibrillation in horses is accompanied with only a slight increase in heart rate most likely related to a high vagal tone (Hnatkova et al., 1998; King et al., 2002; Miller et al., 1999; Silbauer and Sulke, 2007).

AF induced electrical remodeling

Similar to humans, dogs, goats and ponies, AF resulted in a shortening of the AERP and an attenuation of the AERP rate adaptation (Morillo et al., 1995; van Loon et al., 2001b; Wijffels et al., 1995). These changes increase the propensity to develop persistent AF as was observed in two horses. Different theories have been proposed to explain the mechanism of AF primarily based upon ectopic foci, the presence of multiple re-entry waves, the leading circle concept or the presence of rotors and spiral waves (Allessie et al., 1973; Engelman et al., 1986; Jalife et al., 2002; Lewis et al., 1912; Moe, 1962). The sustainability of AF might depend on the possibility of the atrium to maintain a critical number of re-entry circuits (Moe, 1962). This in turn is dependent on (1) the atrial dimensions and on (2) the size of the reentry circuits. The size of the circuit is expressed as the wavelength (WL) and is defined by the

product of the AERP and the conduction velocity (CV) ($WL=AERP \cdot CV$). It is known that if AERP decreases, wavelength decreases and AF will more easily become sustained (Allessie et al., 1985). This phenomenon is amplified by the larger atrial dimensions of the horse compared to goats and ponies. Interestingly the two horses that developed persistent AF had the largest atria.

During this short-term AF protocol, a decrease in AFCL was observed. This decrease might be explained by the shortening of the AERP with the duration of AF or due to a slowing of conduction. Recent data by Allessie (personal communication) however suggest that even during chronic AF, conduction velocity during AF is not decreased.

In the present study we did not investigate the underlying mechanism leading to electrical remodeling but this is most probably related to an increase in intracellular calcium concentration resulting in a reduction of the action potential duration and refractory period (Courtemanche et al., 1998; Allessie et al., 2002).

AF induced contractile remodeling

Twelve hours of maintained AF resulted already in a significant reduction of atrial contractile function that further decreased during the following 7 days. The initial decrease in LA diameter and area at time point “s” might be explained by the small increase in heart rate resulting in a reduced atrial filling time. However, it is unlikely that this phenomenon is completely responsible for a reduction in atrial contractile function (due to reduced filling) since during the next days a further decrease in contractile function is present without further increase in heart rate.

After a short period of induced AF the different $LA_{sx1}/AODV$ ratio's stayed within normal references (<1.2) (Young, 2004). The influence of long standing AF on these ratio's remains to be investigated but data in humans and ponies suggest that chronic AF results in an increase in left atrial size (Sanfilippo et al 1990, van Loon et al., 2001b)

AF induced ventricular remodeling

A short period of atrial fibrillation did not influence ventricular contractile function or ventricular electrophysiology. The latter can be explained by the fact that, in horses, the increase in ventricular rate during AF was very small compared to most other species. Indeed, in human patients, it has been suggested that AF-associated ventricular electrical remodeling was caused by changes in ventricular rate, not by the arrhythmia *per se* (Manios et al. 2005).

Conclusions and clinical implications

In an experimental short-term (1week) AF model, AF leads to rapid electrophysiological and contractile remodeling that might result into self-sustaining, persistent AF in some horses. Upon restoration of sinus rhythm, reverse remodeling occurs within a few days. These observations suggest that early conversion of AF might be beneficial for success rate and early returning to training. However,, in clinical cases, additional factors, such as individual variation or subtle myocardial disease (fibrosis), might complicate the course of AF.

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CHAPTER 2

REMODELING AND REVERSE REMODELING ASSOCIATED WITH LONG-TERM ATRIAL FIBRILLATION

**TIME COURSE OF ATRIAL ELECTRICAL AND
CONTRACTILE REMODELING AND REVERSE
REMODELING AFTER CHRONIC PACING-INDUCED
ATRIAL FIBRILLATION IN HORSES**

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Summary

A previous study has shown that a brief episode (<7 days) of atrial fibrillation (AF) in horses is associated with electrical and contractile remodeling. Reverse remodeling was complete within 2 days. However, little is known about remodeling and reverse remodeling after long-term AF in horses.

In four healthy horses a pacemaker and neurostimulator were implanted. AF was induced with the neurostimulator and maintained by applying intermittent burst pacing for 4 months. Before, during and after the 4-month AF-period, atrial electrophysiology and atrial contractile function were studied.

Significant atrial electrical and contractile remodeling was observed starting from 24-48 hours respectively. Upon restoration of sinus rhythm after 4 months of AF, atrial electrical reverse remodeling was complete within 3 weeks. In contrast, atrial contractility and left atrial diameter only normalized after 2 months.

Four months of pacing-induced AF results in completely reversible electrical and contractile remodeling. However, 2 months are necessary for full recovery of atrial contractile function.

Introduction

Atrial fibrillation (AF) is the most clinically important supraventricular arrhythmia in horses (Deem and Fregin 1982; Reef *et al.* 1988; Reef and McGuirk 2002). This arrhythmia is frequently detected as an incidental finding because symptoms are not always obvious, especially in pleasure horses, although a history of exercise intolerance, exercise-induced epistaxis, respiratory disease, weakness or syncope may be present (Deem and Fregin 1982, Reef and McGuirk 2002). In many cases, AF exists already for several weeks or months before horses are treated. AF duration has been reported to influence the success rate of treatment (Reef *et al.* 1988). In human literature, it is described that AF is a self-promoting electrical disease because of electrical and contractile remodeling, and that successful pharmacological or electrical treatment becomes more difficult the longer AF exists (Gold *et al.* 1986; Alessie *et al.* 2002; Schotten *et al.* 2003). However, little information is available about electrophysiological and contractile changes in horses with chronic AF.

Recent information about electrophysiological and contractile remodeling and reverse remodeling of short-term AF (7 days) in healthy horses has been described (De Clercq *et al.* 2008). In this study, significant atrial electrical and contractile remodeling was observed from 4 and 12 hours onwards, respectively. After restoration of sinus rhythm reverse remodeling was completed within 2 days. This study suggested that horses successfully treated for recent-onset AF only require a short resting period before returning to training. However, because in clinical circumstances many horses suffer from an AF-duration of more than 7 days, information is needed about electrical and contractile remodeling and reverse remodeling of long-term AF. In general, knowledge about AF in horses is gathered from equines with naturally occurring AF. The exact duration of the arrhythmia is usually not known and it is not always clear if these horses have distinct underlying cardiac pathology.

The purpose of the study was to investigate atrial electrophysiological changes and echocardiographic alterations and recovery in healthy horses during a 4-month lasting period of pacing-induced AF and after permanent restoration of sinus rhythm.

Materials and methods

The study was performed at Ghent University (Belgium) and was supported by the Special Research Fund, Ghent University (Belgium). This research was approved by the Ethics Committee of the Faculty Veterinary Medicine, Ghent University (Belgium).

Animal and study protocol

Four healthy trotter horses with a mean age of 4.5 ± 0.6 years, a mean weight of 482 ± 22 kg and a mean height at the withers of 156 ± 3 cm, were studied. A clinical examination, a complete blood analysis, electrocardiography and echocardiography including two-dimensional, M-mode and Colour Flow Doppler, were performed to exclude animals with underlying heart disease.

In all horses, a dual chamber pacemaker and a pulse generator were implanted as previously described by van Loon *et al.* (2002). After implantation a recovery period of 2 months was respected before starting the study.

With the pulse generator, intermittent burst pacing (3 times threshold, 20 Hz, 1s every 2 seconds) was performed to induce bouts of AF. The dual chamber pacemaker allowed studying atrial and ventricular electrophysiology.

The study consisted of 3 study periods: a baseline period, a 4-month lasting AF-period and a 2-month lasting recovery-period. Throughout the study measurements were performed in the unsedated horse. If needed, a temporary pacing catheter was introduced through an introducer sheath (De Clercq *et al.*, 2008b).

During the AF-period, measurements were performed during AF, but also during sinus rhythm. For the latter, under continuous telemetric ECG monitoring, the pulse generator was temporarily switched off to allow sinus rhythm to restore spontaneously. Within 20 minutes after spontaneous restoration of sinus rhythm measurements during sinus rhythm were performed. After termination of the examination, the pulse generator was immediately switched on again for further maintenance of AF.

However, atrial fibrillation became persistent in all horses during the 4-month maintained AF-period. As such, switching off the pulse generator did no longer result in spontaneous restoration of sinus rhythm. The pulse generator was permanently switched off from the time that permanent AF was present. In order to terminate AF at the end of the 4-month AF-period each horse was treated with quinidine sulphate (QS) orally (22 mg/kg by nasogastric intubation every 2 hours) until sinus rhythm occurred, until adverse side effects appeared or when a maximum daily dose of 132 mg/kg was reached.

Electrophysiological measurements

To study the atrial and ventricular effective refractory period (AERP, VERP) a programmed electrical stimulation study was performed at 3 times the baseline threshold amplitude for stimulation. During pacing with a fixed pacing interval (S1-S1) an extra stimulus (S2) was introduced with a coupling interval (S1-S2) below the expected refractory period. The coupling interval was then prolonged in steps of 8 ms until capture of the extra stimulus occurred, i.e. the atrial or ventricular S2 was followed by a P wave or QRS complex on the surface ECG. The longest S1-S2 interval without capture was taken as the AERP or VERP. AERP was measured at pacing cycle lengths (PCL) of 1000 ms (60 beats/min), 800 ms (75 beats/min), 500 ms (120 beats/min) and 333 ms (180 beats/min). VERP was measured at PCL of 1000ms, 800ms and 500ms. A 2-minute interval was respected between each measurement at each pacing cycle length. Refractory periods were measured on 10 separate days during the baseline period, after 1, 2, 3, 4, 7, 14, 21, 45, 60, 90 and 120 days of AF during the AF-period, directly after conversion to sinus rhythm with QS and after 1, 2, 3, 4, 7, 10, 14, 21, 30, 45 and 60 days of the recovery-period.

At the same time intervals before, during and after the AF-period, heart rate and atrial fibrillation cycle length (AFCL) were measured. The AFCL is the time between two successive atrial depolarization waves and was measured from a 10s atrial electrogram.

AF duration was measured as the time between disabling the neurostimulator and spontaneous conversion to sinus rhythm.

Echocardiographic views and measurements

Echocardiographic views were obtained using standardized imaging techniques with a 2.5 MHz sector transducer at a depth of 30 cm (GE Vingmed CFM 800 SV). A single-lead electrocardiogram was recorded simultaneously. Recordings were stored on digital MO-disks and on video tape for retrospective analysis.

Every echocardiographic variable was determined from 5 cardiac cycles to obtain a mean value. All echocardiographic measurements were performed on 5 different days at baseline and after and after 1, 2, 4, 7, 14, 21, 30, 45, 60, 92, 120 days of maintained AF (both during AF and during sinus rhythm) and after 1, 2, 3, 4, 7, 14, 21, 30, 45 and 60 days after termination of the AF-period. Cycles during and immediately after a second-degree atrioventricular block or a spontaneous atrial premature beat were excluded from analysis. During AF, cardiac cycles with an RR interval between 1333 to 1090 ms (45 to 55 beats/min) were selected to make ultrasonographic measurements.

Left atrial (LA) internal diameters (LAD) and LA surface (LAS) were measured from a right parasternal long-axis view. LAD was measured from the interatrial wall, close to the mitral annulus to the atrial free wall (Figure 1). For every cardiac cycle 4 time points were determined to perform these measurements: point “p” was at the onset of the P wave; point “a” was during maximal atrial contraction, point “d” was at the end of the ventricular diastole and point “s” was at the end of ventricular systole. Due to the absence of an atrial contraction, point “p” and “a” could not be determined during AF.

The percentage of left atrial fractional shortening (LA-FS)(%) was calculated with the following formula:

$$LA - FS = \frac{LADp - LADa}{LADp} \times 100$$

The percentage of left atrial fractional area change (LA-FAC)(%) was calculated with the following formula:

$$LA - FAC = \frac{LASp - LASa}{LASp} \times 100$$

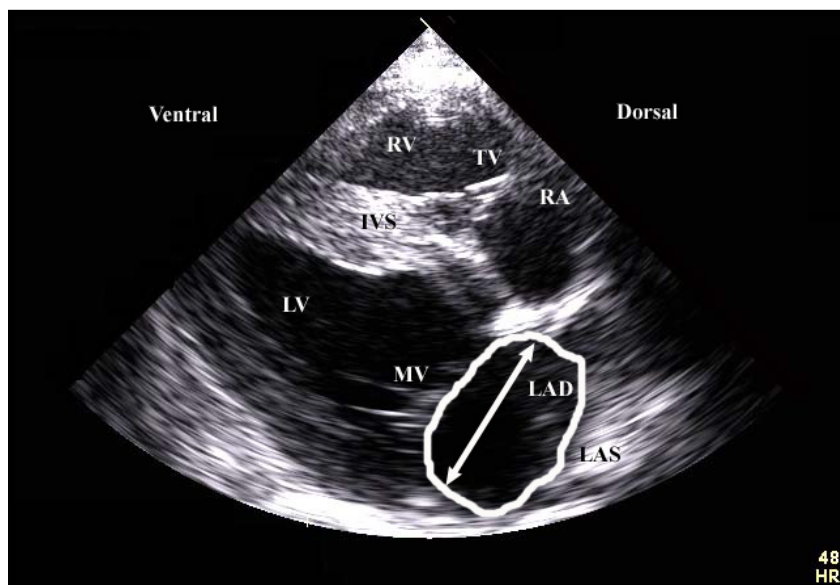
On the right parasternal short-axis view of the LA and aorta, internal diameters of the LA and the aorta were obtained. For the internal short-axis diameter of the LA (LA_{sx}) calipers were placed along a line extending from and parallel to the commissure between the noncoronary and left coronary aortic valve cusps to the distant margin of the left atrium. For the internal short-axis diameter of the aorta (AOD), calipers were placed along the commissure between the noncoronary and right coronary aortic valve cusps. Atrial and aortic measurements performed on the right parasternal short-axis view are illustrated on Figure 2. For each time point (p, a, d, s) during the cardiac cycle the ratio LA_{sx}/AOD was calculated (Young, 2004).

Colour Flow Doppler examinations of the mitral, tricuspid, aortic and pulmonic valves were performed.

Statistical analysis

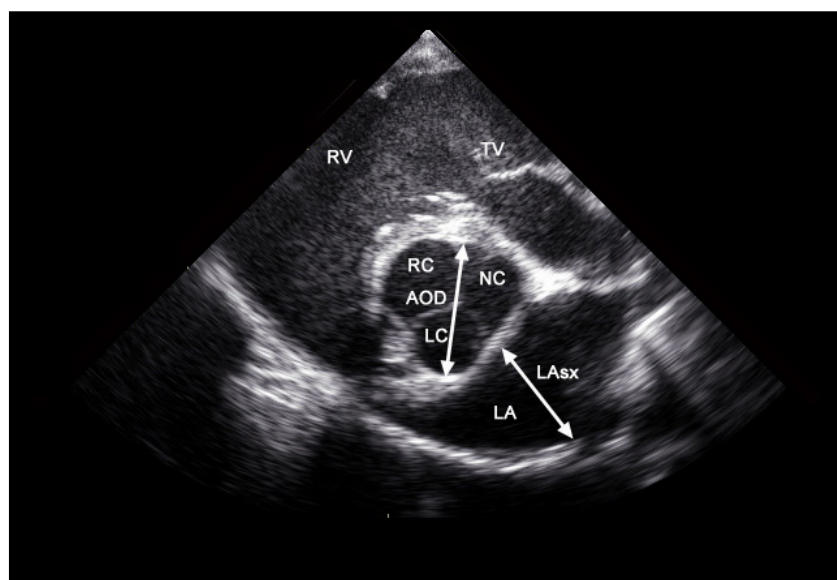
Data are shown as mean \pm standard deviation. All analyses were based on the mixed model with horse as random effect and time as categorical fixed effect. All timepoints were compared with the baseline value at time zero, using Dunnett's method to adjust for multiple comparisons, and a global significance level of 5%.

Figure 1. On the right parasternal long-axis view of a horse, latero-lateral left atrial diameter (LAD) was measured from the lateral free wall to the interatrial septum. The circular line represents the left atrial surface area (LAS). RA: right atrium.



RV: right ventricle. TV: tricuspid valve. LA: left atrium. LV: left ventricle. MV: mitral valve. IVS: interventricular septum.

Figure 2. A right parasternal short-axis view of the left atrium and aorta of a horse. The diameter of the left atrium (LASx) and aorta (AOD) was obtained. To measure the internal short-axis diameter of the aorta calipers were placed along the commissure between the noncoronary and right coronary aortic valve cusps. To obtain the internal short-axis diameter of the left atrium (LASx), calipers were placed along a line extending from and parallel to the commissure between the noncoronary and left coronary aortic valve cusps to the distant margin of the left atrium.



RV: right ventricle. TV: tricuspid valve. LA: left atrium. RC: right coronary aortic cusp. LC: left coronary aortic cusp. NC: non-coronary aortic cusp.

Results

In all horses, AF could be successfully induced and maintained using the intermittent burst pacing protocol. In each horse, threshold for stimulation remained below the upper stimulation capacity (amplitude and duration) of the pacemaker except for horse 2. In this horse, atrial electrical measurements during the recovery period were performed with a temporary pacing catheter (Bipolar Intracardiac Electrode, USCI) introduced through an 8.5F introducer sheath (Intro-Flex, Baxter), placed in the lower half of the jugular vein. Programmed electrical stimulation was performed with an external pacemaker and the pacemaker programmer. At the end of AF period all horses were successfully converted to sinus rhythm after a total of 2 or 3 doses of QS. One horse (horse 2) showed widening of the QRS complex and tachycardia after the 2nd dose of QS.

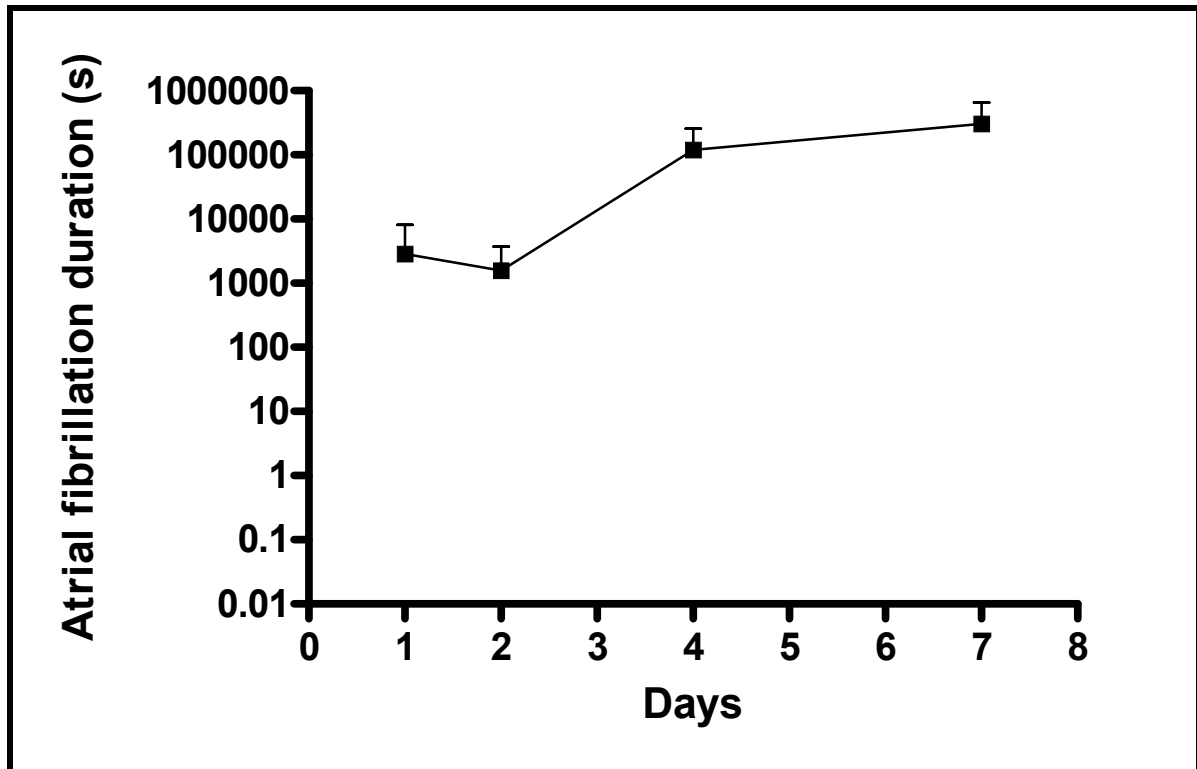
Electrophysiological measurements

Atrial fibrillation

Atrial vulnerability and AF duration were very variable but increased progressively during the AF period and progressed into persistent AF in all horses. AF became persistent after 1 (horse 1 and 3), 4 (horse 2) and 6 (horse 4) weeks of maintained AF (Figure 3). After 4 hours of maintained AF, AFCL was 243 ± 13 ms and decreased to 151 ± 13 ms after 4 months of AF (Figure 4).

There was a significant increase in heart rate during the first week of AF (Table 1).

Figure 3: Mean±SD atrial fibrillation duration (in seconds) after switching off the neurostimulator during the atrial fibrillation study (log scale) of 4 horses.



s: seconds

Figure 4: Mean (±SD) atrial fibrillation cycle length (AFCL) during the AF-period (□) of 4 horses. (■) recovery-period.

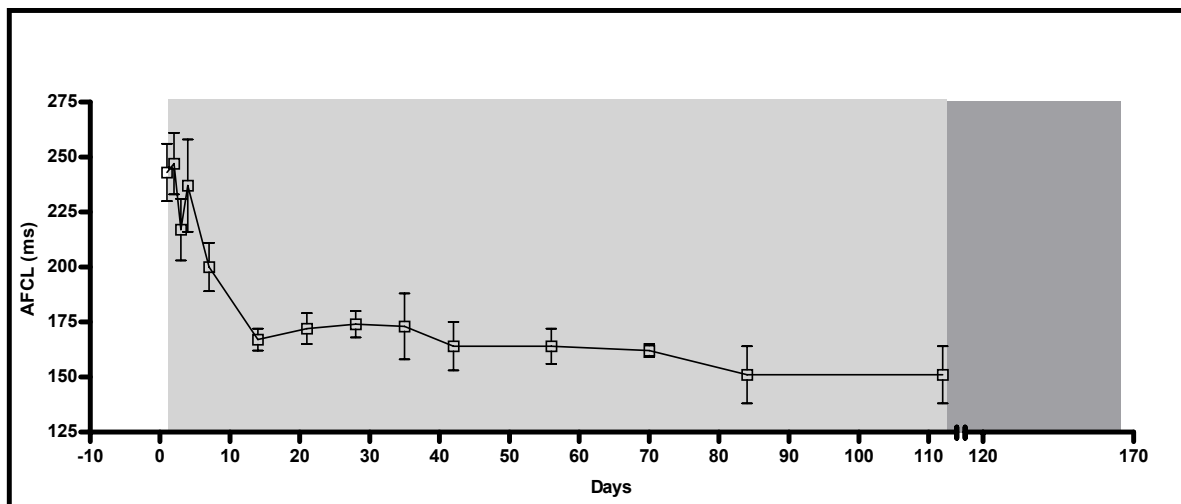


Table 1. Mean (\pm SD) atrial and ventricular electrophysiological and echocardiographic measurements before, and during the AF-period, and after restoration to sinus rhythm of 4 horses.

	BASELINE	AFTER 4 MONTHS OF AF*	MEAN INCREASE %*	BACK TO BASELINE	
				TIME (days)	VALUE
Heart rate (bpm)	40 \pm 3	53 \pm 9	33	3	34 \pm 5 [^]
Electrophysiological measurements					
<i>Right atrium</i>					
AERP ₁₀₀₀ (ms)	256 \pm 24	219 \pm 16	- 14	21	255 \pm 13 [°]
AERP ₈₀₀ (ms)	256 \pm 25	224 \pm 30	- 12	21	255 \pm 11 [°]
AERP ₅₀₀ (ms)	249 \pm 26	248 \pm 30	- 0.5	0	248 \pm 30 [°]
AERP ₃₃₃ (ms)	226 \pm 24	148 \pm 8	- 35	21	225 \pm 12 [°]
<i>Right ventricle</i>					
VERP ₁₀₀₀ (ms)	358 \pm 16	387 \pm 35	8	0	387 \pm 35 [°]
VERP ₈₀₀ (ms)	321 \pm 19	372 \pm 32	16	14	339 \pm 17 [°]
VERP ₅₀₀ (ms)	250 \pm 17	276 \pm 36	10	1	251 \pm 16 [°]
Echocardiographic measurements					
<i>Right parasternal long-axis view</i>					
LA-FS (%)	12.2 \pm 2.5	0.7 \pm 2.0	- 94	14	11.2 \pm 3.4 [°]
LA-FAC (%)	18.9 \pm 6.4	-1.6 \pm 4.2	- 108	14	17.4 \pm 6.2 [°]
LAD _d (cm)	8.7 \pm 0.5	9.1 \pm 0.8	5	7	8.6 \pm 0.6 [°]
LAS _d (cm ²)	41.4 \pm 6.4	50.0 \pm 10.7	21	60	41.6 \pm 7.6 [°]
LAD _s (cm)	9.7 \pm 0.6	9.8 \pm 0.7	1	2	9.7 \pm 0.7 [°]
LAS _s (cm ²)	55.0 \pm 6.4	58.9 \pm 9.9	7	7	55.0 \pm 6.8 [°]
LV-FS (%)	35.4 \pm 3.7	33.7 \pm 4.3	5	2	35.7 \pm 4.3 [°]

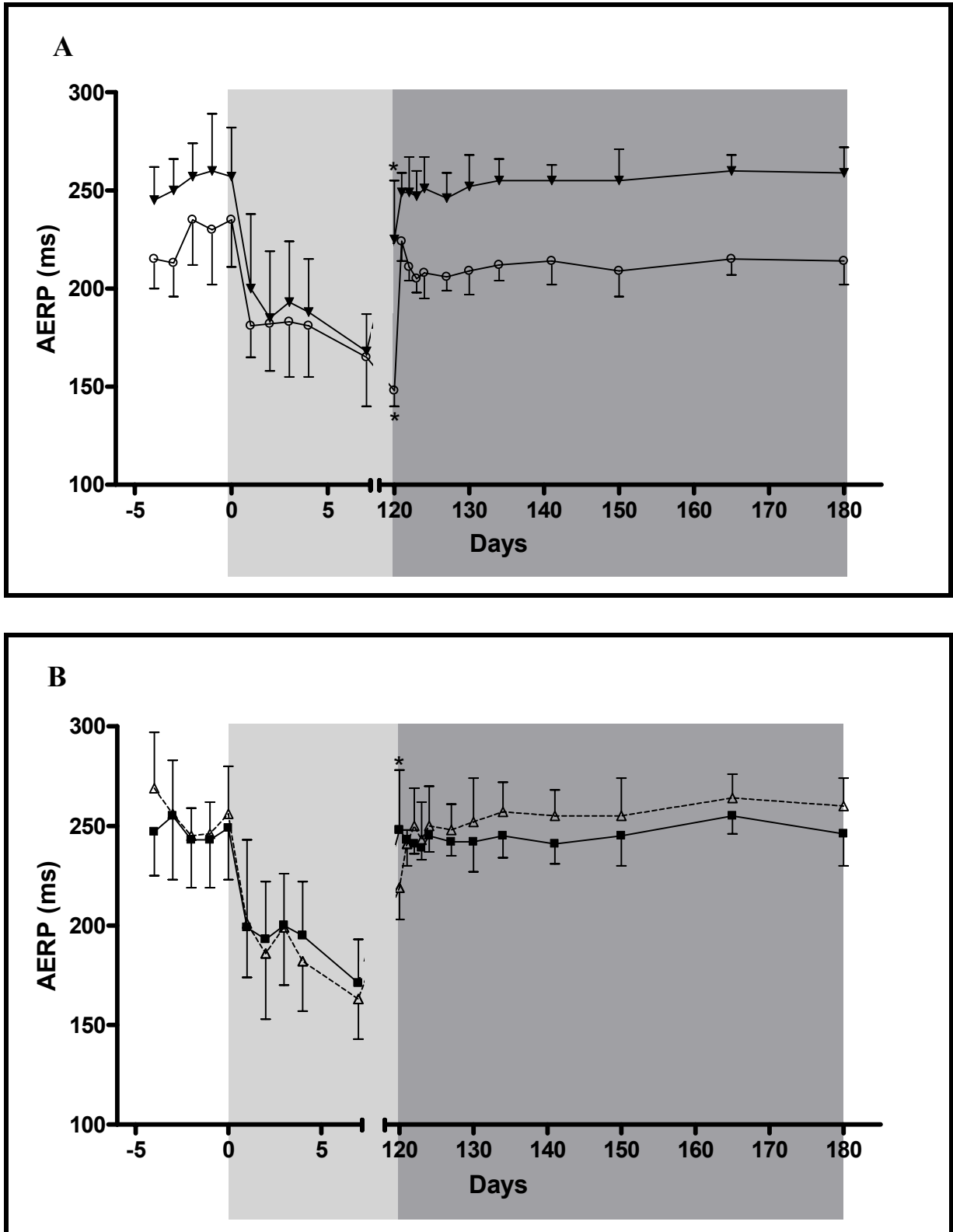
AERP: atrial effective refractory period. VERP: ventricular effective refractory period. LAD: left atrial diameter measured on the right parasternal long-axis view. FS: fractional shortening. FAC: fractional area change. LAS: left atrial surface of the right parasternal long-axis view. d: measurements during ventricular diastole. s: measurements during ventricular systole. LV-FS: left ventricular fractional shortening. *: data obtained after quinidine sulphate treatment. °: data differ less than 10% of baseline value. ^ data differ more than 10% of baseline value.

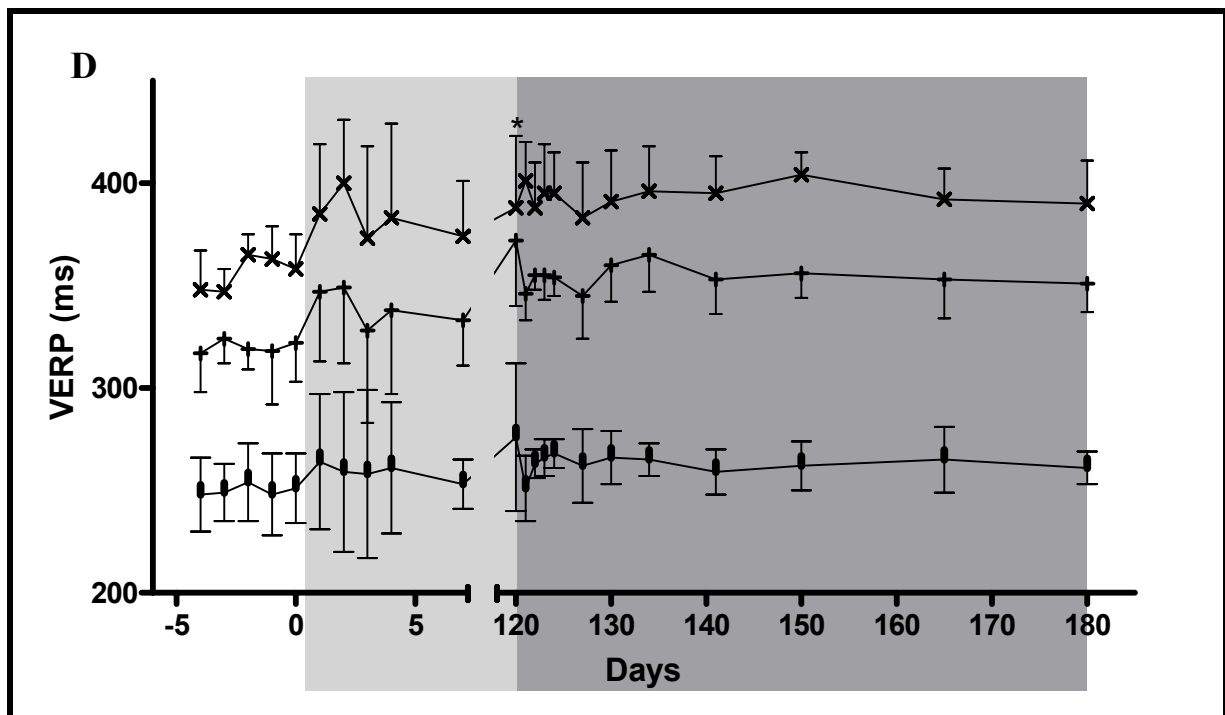
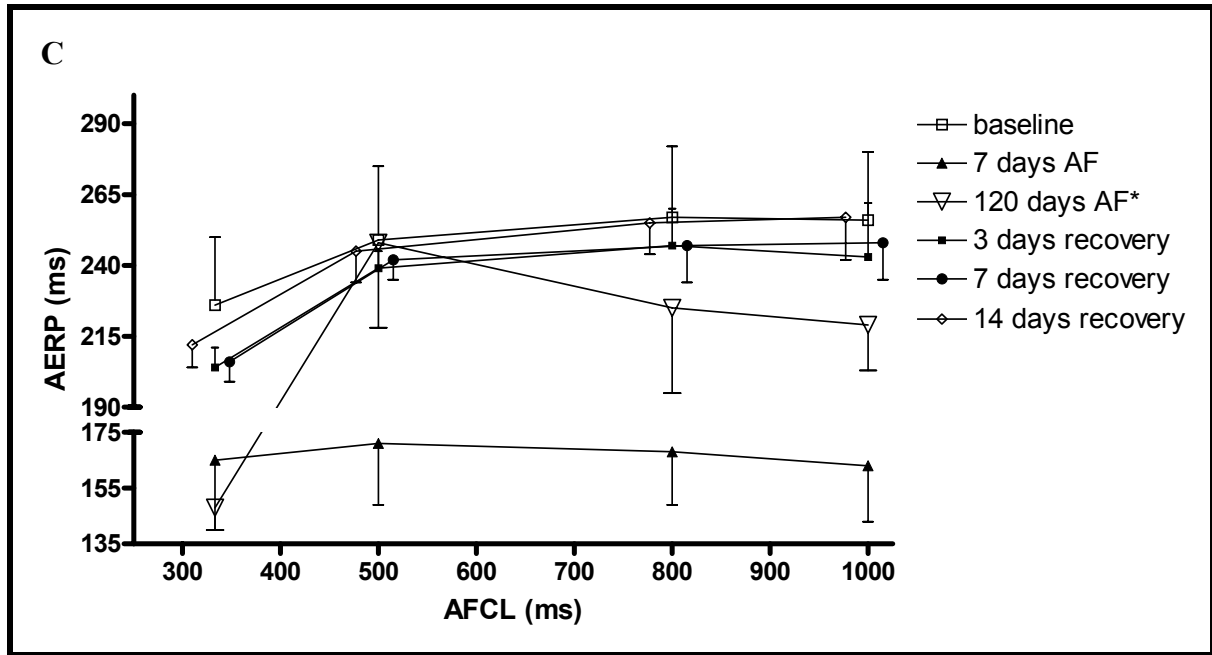
Atrial and ventricular electrophysiology

Results are given in Table 1 and Figure 5. At baseline, AERP was 256 ± 24 ms, 256 ± 25 ms, 249 ± 26 ms and 226 ± 24 ms at a pacing CL of 1000, 800, 500 and 333 ms, respectively (Table 1 and Figure 5 A and B). During the maintained AF-period the AERP shorted significantly from 24 hours onwards for each pacing CL (1000, 800, 500 and 333ms). After seven days of maintained AF, mean \pm SD AERP had decreased to 163 ± 20 , 168 ± 19 , 171 ± 22 and 165 ± 24 ms at a pacing CL of 1000, 800, 500 and 333ms, respectively. A decrease in rate adaptation could also be observed (Figure 5C). After pharmacological restoration to sinus rhythm a period of 3 weeks was necessary to achieve AERP baseline values (Table 1).

No significant changes were observed in VERP (Figure 5D)

Figure 5. Mean (\pm SD) atrial effective refractory period (AERP) at a pacing cycle length of 333 (-O-), 800 ms (- ∇ -) (A) and 1000 (- \blacksquare -), 500 ms (- \triangle -) (B) and ventricular refractory period (VERP) at pacing cycle length of 500 (-|-), 800 (-+ -) and 1000 ms (-X-) (D) at baseline, during the AF-period (□) and during the recovery-time (■) in 4 horses. Rate adaptation curves of the AERP at different time points during the study (C).





*Data obtained after conversion to sinus rhythm with quinidine sulphate.

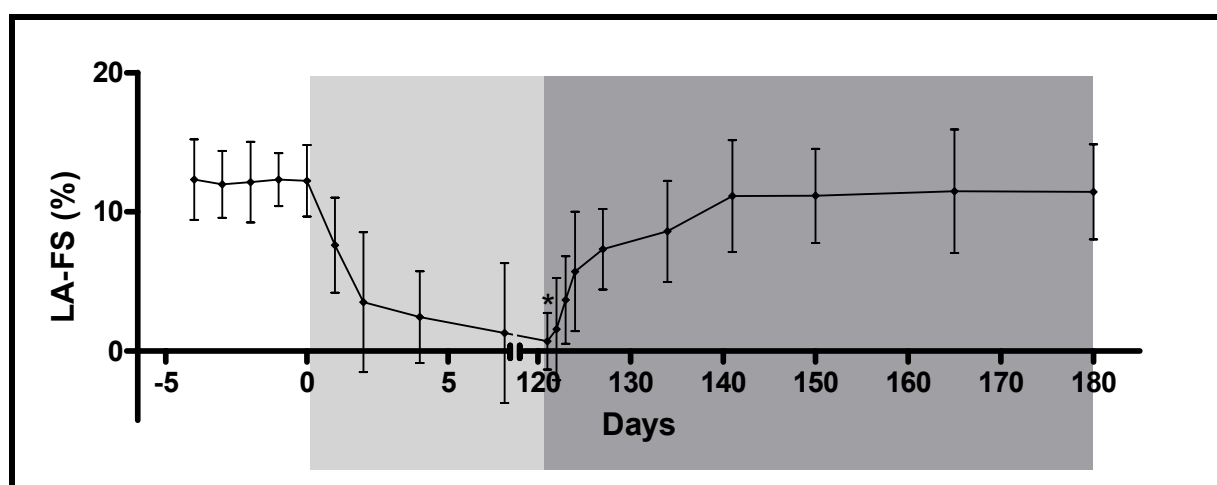
Echocardiographic measurements

Results are given in Table 1 and Figure 6. There were no significant differences between measurements performed during atrial fibrillation and during sinus rhythm in the AF-period. Left atrial FS and FAC decreased significantly within 1 or 2 days of maintained AF ($p < 0.0001$). Atrial contractile function was completely lost within 1 to 4 weeks of maintained AF: a maximum decrease was observed of 94% and 108% compared to baseline for LA-FS and LA-FAC, respectively (Table 1). Both values returned progressively to baseline values but this required 2 months of normal sinus rhythm.

LADd, LADs, LASd and LASs had increased after 4 months of AF with 5%, 1%, 20% and 7%, respectively. All values returned back to baseline values within 2 months. The ratio LA_{sx1}/AOD_{sx} measured at each time point during the cardiac cycle remained below 1.2 (Young 2004). A significant increase for LA_{sxa}/AOD_{sxa} and LAD_{sx1d}/AOD_{sx1d} was observed during the 4-month AF-period. Both values returned back to normal within 7 days of the recovery-period. No significant changes were detected in aortic diameter.

Colour Flow Doppler of all valves revealed no differences compared to baseline.

Figure 6. Course of the overall left atrial fractional shortening (FS%) (mean \pm SD) at baseline, during the AF-period (□) and during the recovery-time (■) (n=6).



Discussion

The present study evaluates electrical and contractile remodeling during chronic maintained AF in horses without underlying structural heart disease. This study population can be considered to be representative for those horses presented with lone subacute or chronic atrial fibrillation. The burst pacing protocol was effective to induce and maintain AF. Besides the measurements made during AF, brief periods of spontaneously restored sinus rhythm allowed making electrophysiological measurements during normal sinus rhythm without any administration of drugs. However, in all horses persistent AF developed after 7, 28, 7 and 42 days for horse 1, 2, 3 and 4, respectively. Therefore, measurements made during the brief periods of spontaneously restored sinus rhythm could only be followed for a short period (7 to 42 days). After 4 months of AF none of the horses converted spontaneously to normal sinus rhythm. We choose for the pharmacological treatment with QS because until now this is still the “gold” standard treatment for chronic atrial fibrillation in horses. The relatively short half-life of quinidine was thought to be beneficial to continue measurements during the recovery period. Horses were not electrically cardioverted because this might influence atrial contractile function (Harjai et al., 1997) and because of the chance for immediate recurrence of AF (De Clercq et al., 2008c).

First of all, the AF study period confirmed the observation made previously: a rather short episode of AF results in (1) electrical remodeling expressed as a decrease in AERP and a depressed AERP rate adaptation curve and (2) contractile remodeling expressed as a decrease in atrial fractional shortening. Atrial contractile dysfunction has also been found in horses with naturally occurring AF (Schwarzwald et al., 2007). Our study also demonstrated that a prolonged period of AF was associated with a minimal increase in atrial size in 4 horses. Despite this increase in diameter, the ratio's LA_{sx1}/AOD_{sx} stayed within the normal references (<1.2) (Young 2004). Besides the shortening of the AERP, a large atrial diameter was more likely to be associated with the development of persistent atrial fibrillation: the two horses that developed persistent atrial fibrillation after one week of maintained AF had the largest atria at baseline (results not shown).

Upon restoration of sinus rhythm rapid electrical reverse remodeling was present but a normalization of atrial contractility and left atrial diameter was only reached after 2 months. These observations are in contrast to the findings made after short episodes of atrial fibrillation (<7 days) where normalization of atrial contractile function is more rapid. These

suggest that after cardioversion of chronic atrial fibrillation in horses a long resting period is required before returning to training.

In summary, the results of this study indicate that AF induces, in healthy horses, a rapid electrical and contractile remodeling and a slow increase in left atrial diameter. In each horse, persistent AF developed after 1 to 6 weeks of intermittent burst pacing. After pharmacological restoration to sinus rhythm, a rapid electrical reverse remodeling and a slower normalization of atrial contractility and left atrial diameter was observed.

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SECTION 2

PHARMACOLOGICAL TREATMENT OF ATRIAL FIBRILLATION IN HORSES

PHARMACOLOGICAL TREATMENT

Until now, *quinidine sulphate* (QS), a class I anti-arrhythmic drug, has been the drug of choice for pharmacological treatment of atrial fibrillation (AF) in horses. It has an efficacy of 85% in horses where AF duration is less than 2 months and where no detectable cardiac disease is present. However, in up to 76% of treated horses this drug is associated with serious cardiac or non-cardiac side effects including urticaria, nasal edema, colic, diarrhea, laminitis, polymorphic ventricular tachycardia, anaphylactic shock, syncope or sudden death. Recently, QS is no longer available in some countries.

In human medicine, a wide range of anti-arrhythmic drugs to treat acute or chronic supraventricular or ventricular arrhythmias. *Propafenone*, a class I anti-arrhythmic drug, is used for treatment of supraventricular and ventricular arrhythmias and several studies describe that this drug is more or as efficient as QS and other class I or class III anti-arrhythmic drugs for prevention and suppression of arrhythmias. *Amiodarone*, a class III anti-arrhythmic drug, is an efficacious and safe anti-arrhythmic drug used in humans to treat AF, atrial flutter and ventricular fibrillation.

Because in horses, intravenous *propafenone* has already been described for treatment of ventricular arrhythmias, the use of this drug in horses with chronic AF was studied in Chapter 3. In Chapters 4 to 6, the use of amiodarone in horses with chronic AF was investigated.

Both studies were performed in horses with chronic AF as most clinical patients are supposed to suffer from chronic AF which is supposed to be more resistant to medical treatment than recent-onset AF.

CHAPTER 3

PROPAFENONE IN HORSES WITH ATRIAL FIBRILLATION

PROPAFENONE FOR CONVERSION OF CHRONIC ATRIAL FIBRILLATION IN HORSES

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Summary

The objective of this study was to investigate the effects of intravenous propafenone in horses with naturally-occurring and experimentally-induced chronic atrial fibrillation (AF). The study was performed on two horses with naturally-occurring chronic (6 to 12 months) AF and 4 horses with pacing induced chronic (4 months) AF. The mean duration of spontaneous self-sustained AF in the latter group, was 3 months.

All horses received a 2 mg/kg propafenone bolus IV over 15 minutes. If AF persisted after 20 minutes, a continuous infusion of 7 mcg/kg/min of propafenone was given for 120 minutes. Before, during and after the treatment protocol, propafenone concentrations, haematological and biochemical analyses and electrolyte concentrations were determined and clinical signs were monitored. At regular intervals surface electrocardiograms, for determination of heart rate, f wave interval, QRS duration and QT interval, and intra-atrial electrograms, for measurement of atrial fibrillation cycle length (AFCL) were recorded. If propafenone treatment failed, quinidine sulphate was administered.

All horses received both the bolus and continuous infusion with minimal adverse effects. During the 15-minute bolus a slight increase in heart rate was observed and horses appeared more sensitive to external stimuli. Throughout the treatment, no significant changes were observed in respiratory rate, QRS or QT duration and blood analysis remained within normal references. Although a highly significant ($p < 0.001$) increase in f wave interval and AFCL was observed and propafenone concentrations (569-1268 ng/mL) reached the human therapeutic level (64-1044 ng/mL), none of the horses cardioverted to sinus rhythm. Sinus rhythm could be restored in all horses with a standard, oral QS treatment.

Although the current propafenone treatment resulted in therapeutic propafenone concentrations without important side effects, this drug failed to restore sinus rhythm in any horse with chronic AF.

Introduction

Atrial fibrillation (AF) is the most important arrhythmia in horses. Treatment is recommended in horses with AF in the absence of underlying heart disease because the horse's performance generally returns to the previous level after restoration to sinus rhythm. In horses, atrial fibrillation may be detected as an incidental finding because symptoms are not always obvious, especially in pleasure horses. However, a history of exercise intolerance, exercise-induced epistaxis, respiratory disease, weakness or syncope may be present (Deem and Fregin, 1982; Reef and McGuirk, 2002). In many cases, history suggests that AF is already present for several weeks or even months before horses are presented for cardioversion. It has been suggested that when AF persists for >3-6 months or when atrial dilatation is present because of mitral valve regurgitation or AF *per se*, treatment is less effective (Reef et al., 1988; van Loon et al., 2001a;2001b).

Oral quinidine sulphate, a class I anti-arrhythmic drug, is the drug of choice for pharmacological treatment of acute and chronic AF in horses. An efficacy of 85% in horses with an AF duration of < 2 months and without the presence of detectable cardiac disease has been reported (Deem and Fregin, 1982; Reef et al. 1988). Nevertheless, in up to 76% of treated horses this drug can yield serious cardiac and non-cardiac side effects including urticaria, nasal oedema, colic, diarrhea, laminitis, polymorphic ventricular tachycardia, anaphylactic shock, syncope or sudden death (Reef et al., 1988; 1995). In addition, in some countries quinidine sulphate is no longer available.

Although intravenous flecainide, a class I anti-arrhythmic drug, was originally reported to be efficacious in acute (experimentally induced) AF (Ohmura et al., 2000), life threatening and even lethal ventricular dysrhythmias have been reported in both acute (unpublished results) and chronic AF horses (van Loon et al., 2004). Intravenous cibenzoline (0.1 mg/kg/min), a class I anti-arrhythmic drug (with additional class III and IV properties), was unsuccessful in 2 horses with AF and was associated with severe ventricular pro-arrhythmia (van Loon, 2003).

Sotalol, a class III drug, has been used in three horses with AF (0.75-1.25 mg/kg IV infusion over 15 minutes) and was not associated with side-effects. However, sinus rhythm could not be restored (van Loon, 2003).

Intravenous amiodarone, a class III anti-arrhythmic drug, has been described in horses with chronic atrial fibrillation but with a moderate success rate of 50-67% and occurrence of important side effects, especially with prolonged (> 36 hours) protocols (De Clercq et al., 2006, 2007a).

Successful electrical cardioversion has been described for horses with AF (Deem and Fregin, 1982; van Loon, 2001b; Frye et al., 2002; McGurrin et al., 2003, 2005a, 2005b; van Loon et al., 2005; De Clercq et al., 2007b). However, this technique requires general anaesthesia, special equipment and expertise which may make the process more expensive.

Further research for alternative pharmacological treatment options might be necessary and useful. Kochiadakis et al. (1999) reported that propafenone appeared to be safe and moderately effective for the treatment of chronic atrial fibrillation in humans.

This article describes the effects of an intravenous propafenone treatment in horses with naturally-occurring or experimentally-induced chronic AF.

Material and Methods

The study was performed at Ghent University (Belgium) and was supported by the Special Research Fund, Ghent University (Belgium). This research was approved by the Ethics Committee of the Faculty Veterinary Medicine, Ghent University.

Cases

Six trotter horses with a mean age, body weight and height at the withers of 5.6 ± 2.1 years, 517 ± 65 kg and 158 ± 7 cm, respectively, were studied. Two of the six trotter horses (Horse 1 and 2) had a history of exercise intolerance and naturally-occurring AF of duration of six months and one year. In the 4 other horses (horse 3-6), AF had been experimentally induced and maintained as described by van Loon et al. (2001) during a 4 month period. In these horses a transvenous screw-in electrode was implanted in the right atrium and connected with a pacemaker to deliver repeated bursts of electrical stimuli to the atrium. Initially, cessation of burst pacing resulted in relative short episodes of AF. As burst pacing was continued, the induced AF episodes increased and became self-sustained in all horses. At the time of propafenone treatment, the mean duration of spontaneous, self-sustained AF was 3 months. At that time AFCL was 138 ± 15 ms, compared to 138 ± 6 ms in the horses with naturally-occurring AF.

Treatment regime

Horses received 2 mg/kg of propafenone (Rhytmonorm®, 70 mg/20mL, Abbott) IV over 15 minutes. If AF persisted 20 minutes after this bolus, a continuous infusion of 7 mcg/kg/min of propafenone was given for 120 minutes. Infusion was discontinued when conversion was achieved, when any side effects were observed or the 120-minute infusion was completed.

Recorded data

Before propafenone treatment clinical examination, a base-apex electrocardiogram and echocardiography were performed in all horses. Echocardiography included two-dimensional, M-mode and colour flow Doppler. The two-dimensional and M-mode measurements from the left cardiac window included left atrial diameter during ventricular diastole and systole and, from the right cardiac window, aortic diameter in diastole, left ventricular internal diameter in diastole and systole, interventricular septum thickness in diastole and systole and left

ventricular fractional shortening. Measurements were performed by one observer before and at 2.5, 5, 7.5, 10, 12.5, 15, 35, 60, 90, 120, 150 and 180 minutes of the study protocol. Before, during and after propafenone treatment clinical signs were monitored and intra-atrial electrograms and surface ECG's were recorded. From the surface ECG heart rate (HR), f-wave interval (ms), QRS duration (ms) and corrected QT interval (ms) were measured. The atrial fibrillation cycle length (AFCL) was measured with a pacemaker and a pacemaker programmer (Programmer 9790, Medtronic) as described by van Loon et al. (2000). In these horses a transvenous screw-in electrode had previously been implanted in the right atrium and was connected to an implantable pacemaker. The pacemaker programmer allowed us to measure directly the AFCL on an intra-atrial electrogram. In the non-instrumented horses an 8.5F introducer sheath (Intro-Flex, Baxter) was placed in the lower half of the jugular vein. In the standing and non-sedated animal, a temporary pacing/sensing catheter (U.S.C.I., Bard, Ireland) was introduced through the introducer sheath and placed under echocardiographic guidance in the right atrium. Atrial endocardomyocardial contact of the temporary pacing sensing catheter resulted in rapid atrial deflections on the intra-atrial electrogram. The mean interval between the f waves and the AFCL were measured from a 2 to 10 s window.

Blood samples for retrospective propafenone concentrations were taken at baseline and at 2.5, 5, 7.5, 10, 12.5, 15, 35, 60, 90, 120, 150, 180, 480, 720 and 1440 minutes of the protocol. The plasma concentrations were analyzed by a validated high performance liquid chromatography combined with UV detection (Verbesselt et al., 1991). A limit of quantification of 5 ng/mL was obtained.

Blood samples for haematological and biochemical analyses including white blood cell count, differential analysis, total bilirubin, creatinine, total protein, urea nitrogen, alkaline phosphatase, aspartate aminotransferase, creatinine kinase, gamma-glutamyl transferase, lactate dehydrogenase, and electrolyte concentrations were taken at baseline, after the 15-minute bolus and 24 hours after cessation of the propafenone treatment.

Statistical analysis

Effects of the propafenone treatment on respiratory rate, heart rate, AFCL, f wave interval, QRS duration and corrected QT interval were analysed using single-factor ANOVA. A Dunnett's test was performed to detect possible significant differences compared to baseline values ($p < 0.05$).

Results

All electrolyte concentrations and haematological, biochemical parameters were within normal references. Cardiac evaluation performed by auscultation, echocardiography (2D and M-mode) and Colour Flow Doppler were normal, except for horse 2 which had a mild mitral valve regurgitation. Left atrial diameters during diastole and systole were within the reference ranges (Patteson et al., 1995).

Every horse received the full treatment protocol. During the 15-minute bolus each horse was more sensitive to external stimuli. No other cardiac or non-cardiac adverse effects were observed. This minimal excitation disappeared gradually within 30 minutes after termination of the 15-minute bolus. Cardioversion to sinus rhythm could not be achieved.

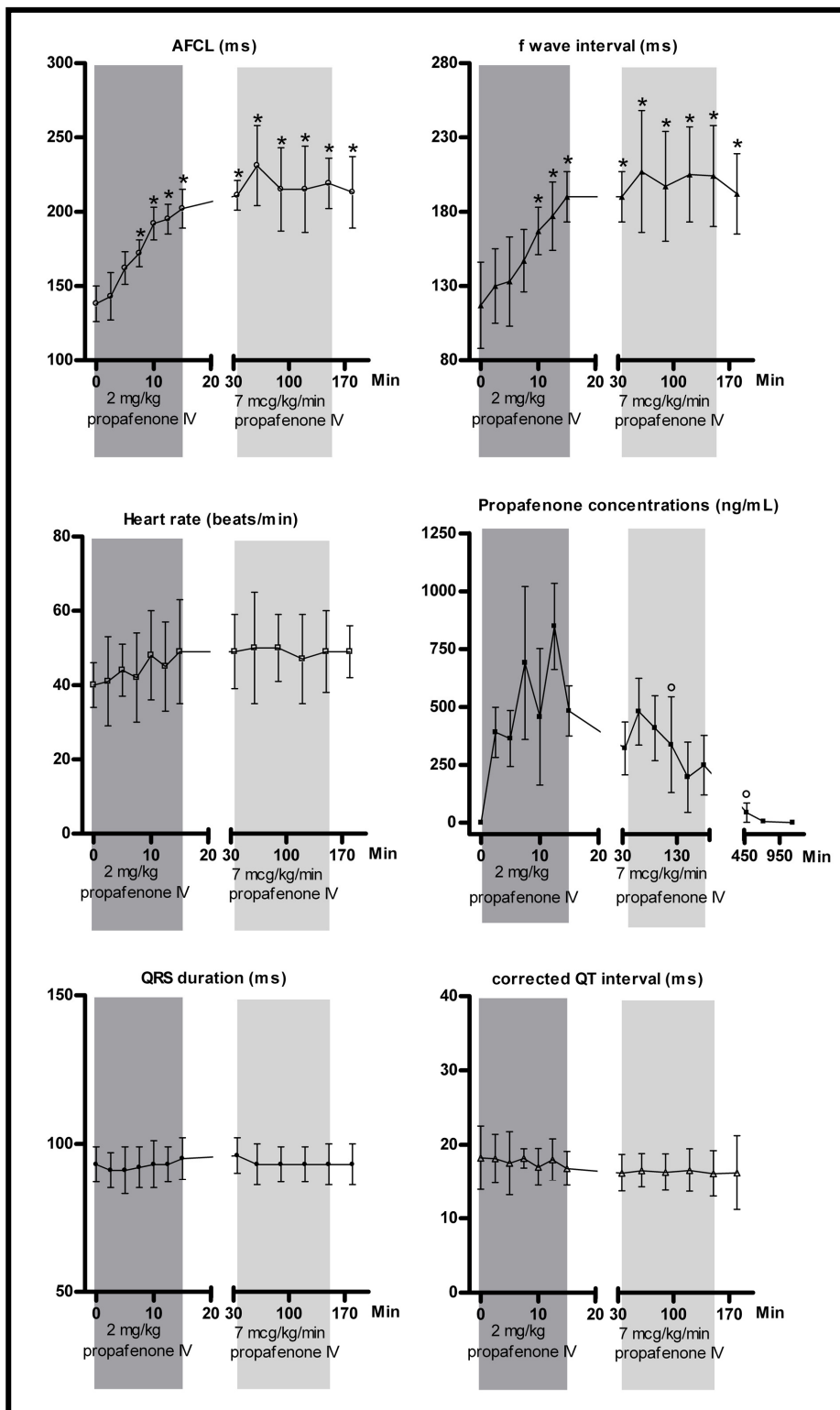
From the numerical description of the data for respiratory rate, HR, AFCL, f wave interval, QRS duration and corrected QT interval as well as from the error bar graphs, it was concluded that the condition of equality of variances was satisfied. For the respiratory rate, HR, QRS duration and corrected QT interval, statistical analysis did not reveal significant differences ($p < 0.05$). However, a very slight increase in heart rate was observed (Figure 1). The ANOVA revealed a significant difference in the mean values over time for AFCL and f wave interval from 7.5 and 10 minutes onwards ($P < 0.05$), respectively, and a highly significant difference from 10 and 12.5 ($P < 0.01$), respectively (Figure 1). However, although an increase in AFCL could be observed in all horses, no cardioversion could be achieved.

Propafenone concentrations (ng/mL) during and after the treatment are displayed in Figure 1. Two plasma samples (at 120 and 480 minutes) of horse 6 were destroyed during centrifugation and no data of these time points were available.

Haematological and biochemical parameters remained within normal limits after the 15 minute bolus and twenty four hours after cessation of the propafenone treatment.

One week after propafenone treatment the four instrumented horses and one horse (horse 1) with naturally occurring AF were treated with oral QS. Each horse converted successfully to sinus rhythm after 2 or 3 doses of QS. One horse showed tachycardia and QRS widening. Thirty minutes before restoration to sinus rhythm, the mean (\pm SD) f wave interval was 376 (\pm 46) ms and QRS duration was 108 (\pm 11) ms.

Figure 1. Mean (\pm SD) atrial fibrillation cycle length (AFCL), f wave interval, heart rate (HR), propafenone concentrations, QRS duration and corrected QT interval (n=6) before, during and after the propafenone treatment.



* significantly different from baseline value.

° data obtained from 5 horses.

Discussion

Propafenone, a class I anti-arrhythmic drug is used successfully to convert atrial fibrillation of recent onset in humans with a success rate of up to 83% (Khan et al., 2001; Fuster et al., 2006). As with other conventional anti-arrhythmic drugs, its efficacy in long lasting AF is lower. For different species a great intersubject variability in elimination half-life of propafenone is described. The elimination half-life after a single intravenous propafenone bolus is about 150, 83 and 120 minutes for humans, dogs and horses, respectively (Arboix et al., 1985; Connolly et al., 1983; Hollmann et al., 1983; Puigdemont et al., 1987; 1990). Based upon these observations we investigated the potential of intravenous administration of propafenone at a dose of 2 mg/kg to treat horses with chronic AF. At the time of propafenone treatment, the mean duration of spontaneous self-sustained AF in the instrumented horses was 3 months. For the horses with experimentally-induced AF, AF induction by burst pacing was only applied for a few weeks. For the remainder of time, AF continued spontaneously without any further intervention. As such, AF in these horses was thought to resemble naturally-occurring chronic AF. In addition, AFCL in both groups was similar (138 ± 15 versus 138 ± 6). In contrast to humans, in our study none of the animals converted to sinus rhythm.

The doses used in our treatment protocol were those described for humans adapted to the weight of a horse (Capucci et al., 1992; Kochiadakis et al., 1999; Lavanga et al., 1991).

In humans, therapeutic propafenone plasma concentration is very variable and ranges between 20-60 ng/mL (Steurer et al., 1991), 50-200 ng/mL (Chan et al., 1989) and 64-1044 ng/mL (Connolly et al., 1983). Steurer and co-workers (1991) and Karagueuzian and co-workers (1982), described a decreased propafenone efficacy at a plasma concentration below 20 ng/mL in humans and below 500 ng/mL in dogs. In humans, ranges above 4180 ng/mL are described as being lethal (Clarot et al., 2003). However, intersubject variability in therapeutic concentration emphasizes the need to individualize therapy to the patient (Connolly et al., 1983). A pharmacokinetic study of propafenone in horses described a rapid elimination half-life (1-2 hours) after a single bolus of 2 mg/kg (Puigdemont et al., 1990). However, injection rate was not mentioned and clinical signs, surface electrocardiograms or other values were not followed. Because of the rapid decline in plasma concentrations, these authors suggested the use of a continuous infusion for administration of the drug without mentioning a dose protocol (Puigdemont et al., 1990). With our treatment protocol (2 mg/kg), a dosage higher

than suggested for the management of ventricular arrhythmia (Reef, 1999), horses reached the human lower therapeutic level of 20-500 ng/mL.

However, this resulted in a highly significant prolongation of AFCL ($p < 0.001$), suggesting a significant slowing of conduction at the atrial level at this propafenone concentration. This effect was obtained without significant adverse reactions. The administration of a higher dosage of propafenone could result in a further increase of AFCL but with a higher risk of major side effects. Flecainide, another class Ic anti-arrhythmic drug, induced a prolongation of the AFCL to a median of 200 ms and a prolongation of the QRS complex to 130 ms. This was associated with the development of serious potentially life-threatening ventricular arrhythmia (van Loon et al., 2004). Furthermore we observed in one horse (unpublished data) the development of ventricular tachycardia with very wide QRS complexes leading to ventricular fibrillation and asystole in a dosage of flecainide of 2 mg/kg. The preceding QRS duration was 140 ms and AFCL was 286 ms. Based upon these observations we limited the dosage of propafenone to 2 mg/kg in order to avoid QRS prolongation, an early marker for the development of ventricular proarrhythmia.

In contrast to propafenone, quinidine sulphate treatment resulted in the termination of AF in all our horses. The degree of AFCL prolongation was much more pronounced after quinidine compared to propafenone. This results in an increased reduction of the average number of fibrillation waves increasing the statistical change of termination of AF (Allessie et al., 1998).

In human medicine, side effects due to acute or chronic doses of propafenone are cardiac, hepatic, cholestatic, dermatologic or nervous disorders (Cocozzella et al., 2003; Gandolfi et al., 2001; Huang et al., 2005; Odeh et al., 2000; Spinler et al., 1992; Wiesfeld et al., 2006). In case of cardiac adverse reactions, some authors described successful therapy with administration of alkalinising solutions (e.g. bicarbonate) or rifampicine in order to increase the metabolism of propafenone (Brubacher, 2004; Stancak et al., 2004; Unal et al., 2007). We did not observe any significant side effect of propafenone in the given dose in these horses.

In conclusion, an IV propafenone treatment in horses with naturally-occurring or induced chronic AF resulted in significant electrophysiological right atrial changes but failed to reverse AF into sinus rhythm. Overall, the value of class Ic anti-arrhythmic drug in the termination of AF in horses seems limited.

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CHAPTER 4

AMIODARONE IN HORSES WITH CHRONIC ATRIAL FIBRILLATION

INTRAVENOUS AMIODARONE TREATMENT IN HORSES WITH CHRONIC ATRIAL FIBRILLATION

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Summary

Six horses without underlying cardiac disease were presented because of atrial fibrillation of between 5 and 12 months duration. These horses received an intravenous amiodarone treatment of 5 mg/kg/h for 1 h followed by 0.83 mg/kg/h for 23 h and subsequently 1.9 mg/kg/h for 30 h. During treatment, clinical signs were monitored and a surface ECG and an intra-atrial electrogram were recorded. Infusion was discontinued when sinus rhythm or side effects occurred. Four horses successfully cardioverted, of which one showed symptoms of hind limb weakness and weight shifting. Two horses did not cardiovert and showed similar side effects. In all horses, side effects disappeared within 6 hours after termination of treatment. Cardiac side effects, such as pro-arrhythmia, were not seen in any of the horses. Total bilirubin slightly increased in 3 horses and normalized within 4 days. It was concluded that amiodarone has the potential to treat naturally-occurring chronic atrial fibrillation in horses, although further research is needed to refine the infusion protocol.

Introduction

Atrial fibrillation (AF) is the most important cardiac arrhythmia in horses. Although some horses have a history of exercise intolerance, exercise-induced epistaxis, respiratory disease, weakness or syncope, AF may be detected as an incidental finding in an otherwise normal horse (Deem and Fregin, 1982, Reef et al., 1988 and Reef and McGuirk, 2002). Horses are predisposed to AF because they have a high resting vagal tone and a large atrium. In contrast to humans and dogs, horses usually have no macroscopic underlying cardiac pathology. This means that after successful medical treatment these animals can return to their previous athletic ability (Reef et al., 1988).

Quinidine sulphate (QS) is used worldwide to treat AF in horses but may result in anaphylactic shock, colic, diarrhea, laminitis, tachycardia or sudden death (Deem and Fregin, 1982; Marr et al., 1995; Reef et al., 1988; Reef et al., 1995; Reef and McGuirk, 2002). Because QS becomes increasingly difficult to obtain, and is expensive, there is a need to find an alternative and preferably less toxic treatment for chronic AF in horses (McGurrin et al., 2003). Flecainide, a Na⁺-channel blocker has been shown to be efficacious in horses with acute AF (Ohmura et al., 2000) but not in horses with chronic AF. It can induce potentially dangerous ventricular dysrhythmias (van Loon et al., 2004).

In this report the use of intravenous (IV) amiodarone (AD), mainly a potassium-channel blocker, as a treatment of chronic AF in horses is described. The drug is considered as a class III compound in the “*Vaughan–William*” classification: but in addition it blocks sodium-, calcium-channels and β -adrenoreceptors, therefore it prolongs repolarization and increases the duration of the action potential (Gill et al., 1992).

Materials and methods

Cases

Five Warmblood horses and one Standardbred (horse 1) were referred for arrhythmia (horses 4 and 6), exercise intolerance (horse 1), epistaxis (horse 5), colic (horse 2) and castration (horse 3), and were each diagnosed with AF. Their mean age, body weight and height at the withers were 11 years (3.5–17.0 years), 602 kg (520–647 kg) and 170 cm (153–180 cm). All horses, except horse 3, were used for trotting, jumping or pleasure riding and all except horse 3 had a history of exercise intolerance, respiratory symptoms or nose bleeding. Based upon history and clinical examination, the duration of atrial fibrillation was thought to have been present chronically for between 5 and 12 months. Horse 6 had been treated 4 weeks previously with QS but after 4 doses the horse had shown severe colic symptoms that necessitated termination of treatment.

A clinical examination and a complete blood analysis (Spotchem SP-4420) were performed. A full cardiac evaluation, including auscultation, ECG, two-dimensional, M-mode and Colour Flow Doppler echocardiography (GE Vingmed CFM 800 SV), was also undertaken (Table 1).

Table 1. Auscultation of the heart, echocardiographic (2D, M-mode and Colour Flow Doppler) and intra-atrial measurements before treatment in 6 horses.

	Horse 1	Horse 2	Horse 3	Horse 4	Horse 5	Horse 6
Auscultation						
auscultation	no murmurs	1/6 holosystolic over TV	1/6 holosystolic over MV	no murmurs	1/6 holosystolic over TV	no murmurs
Echocardiography (2D and M-mode)						
LADd (cm)	11.30	13.31	14.41	15.66	14.43	15.25
LADs (cm)	12.33	13.74	14.77	15.99	14.96	15.55
Ao (cm)	6.24	6.18	6.17	6.97	7.15	7.02
LVIDd (cm)	12.07	10.80	12.64	13.10	12.07	11.15
LVIDs (cm)	7.93	6.21	8.85	7.93	7.51	7.47
FS (%)	34.29	42.55	30.00	39.47	37.78	32.99
Colour Flow Doppler						
mitral valve	no	no	trivial	no	no	trivial
aortic valve	no	no	no	no	no	trivial
pulmonary valve	no	no	no	no	no	moderate
tricuspid valve	no	trivial	no	no	trivial	no
Intra-atrial electrogram						
AFCL (ms)	167	160	148	143	182	174

LADd: left atrial diameter in diastole. LADs: left atrial diameter in systole. Ao: aortic diameter.

LVIDd: left ventricular internal diameter in diastole. LVIDs: left ventricular internal diameter in systole.

FS: left ventricular fractional shortening. AFCL: atrial fibrillation cycle length. no: no insufficiency.

TV: tricuspid valve area. MV: mitral valve area.

Treatment regimen

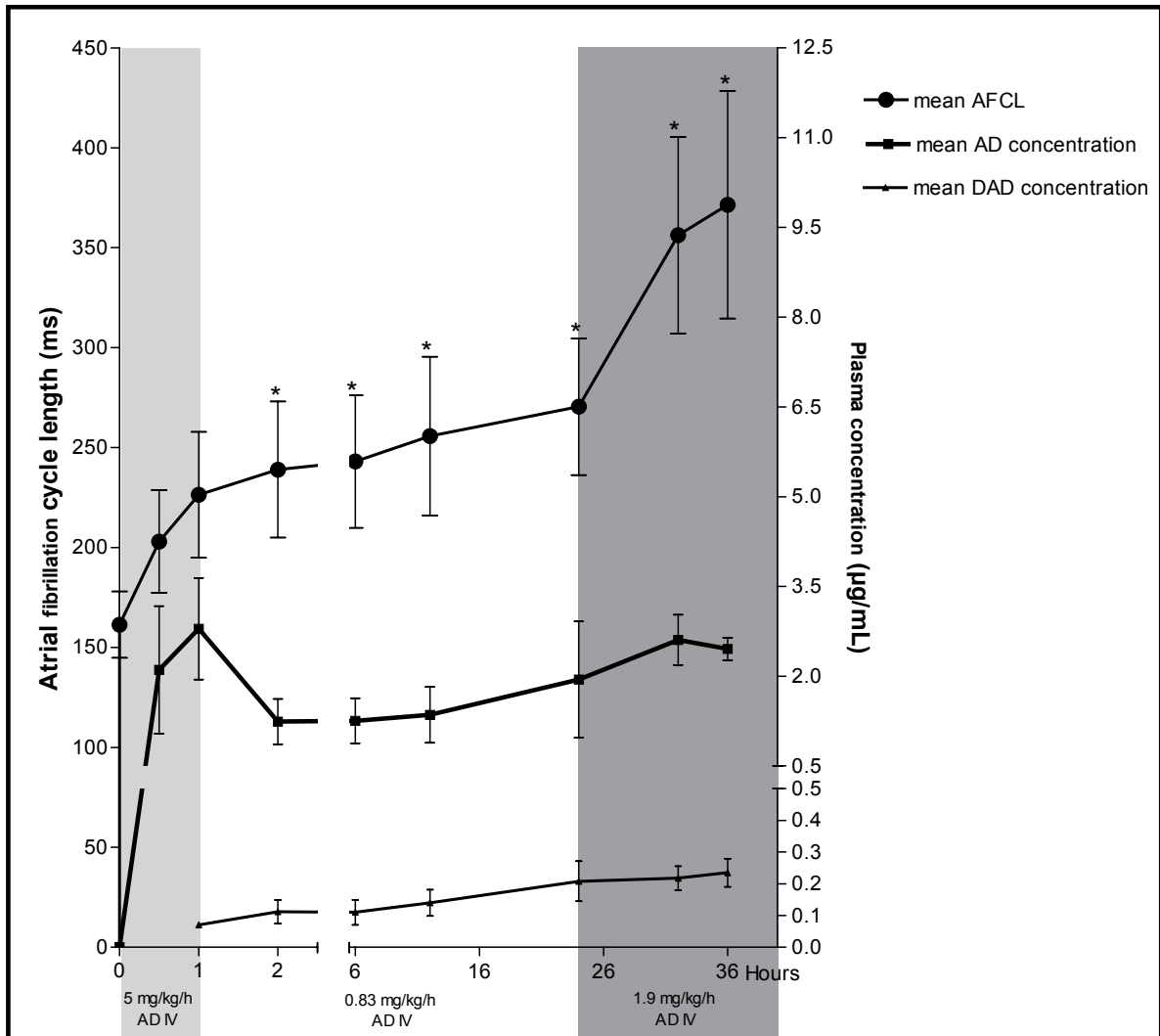
Horses received 5 mg/kg/h AD (Cordarone®, 150 mg/3 mL, Sanofi-Synthélabo) IV over 1 hour followed by 0.83 mg/kg/h AD IV for 23 hours and then 1.9 mg/kg/h AD IV for the following 30 hours. Infusion was discontinued when conversion was achieved or when any side effects were observed. Horses that failed to convert to normal sinus rhythm with AD IV were treated after 1 week with 22 mg/kg quinidine sulphate (QS) administered by nasogastric intubation every 2 hours until sinus rhythm occurred, or adverse side effects appeared, or until a maximum daily dose of 132 mg/kg was administered.

Recorded data

Two-dimensional and M-mode echocardiographic measurements included left atrial diameter in diastole and systole measured from the left cardiac window, aortic diameter in diastole, left ventricular internal diameter in diastole and systole and left ventricular fractional shortening from the right cardiac window (Patteson et al., 1995; Patteson, 1999). Colour Flow Doppler echocardiography was used to identify valvular regurgitation.

Before and during amiodarone treatment clinical signs were monitored and ECGs were recorded. From the surface ECG heart rate, the interval between f waves (ms), QRS duration (ms) and QT duration (ms) were measured. With a temporary pacing catheter (USCI, Bard) placed in the right atrium, the local atrial electrogram was recorded using a pacemaker (Thera D, Medtronic) and a pacemaker programmer (Programmer 9790, Medtronic). From this electrogram the atrial fibrillation cycle length (AFCL), which is the time between two successive atrial depolarization waves, was recorded (van Loon et al., 1998). The mean interval between the f waves and AFCL were estimated from a 2 to 10 s window (Figure 1 and Table 2).

Figure 1. Mean atrial fibrillation cycle length (AFCL, n=6) in ms, amiodarone (AD, n=5) and desethylamiodarone (DAD, n=5) plasma concentration ($\mu\text{g/mL}$) with standard deviation, in the different patients during treatment.



IV: intravenous.

* statistical significant from baseline value.

Table 2. Measurements on the surface ECG (f wave interval) and intra-atrial electrogram (AFCL) and amiodarone (AD) and desethylamiodarone (DAD) plasma concentrations for each horse.

	Time (hours)	AD ($\mu\text{g/mL}$)	DAD ($\mu\text{g/mL}$)	AFCL (ms)	f wave interval (ms)	
Horse 1	0	/	/	167	180	
	0.5	/	/	190	180	
	1	/	/	222	180	
	2	/	/	222	180	
	6	/	/	222	200	
	12	/	/	222	200	
	24	/	/	250	220	
	28	/	/	267	240	
	36	/	/	286	260	
	48	/	/	400	260	
	54	/	/	400	260	
69	/	/	SR	SR		
Horse 2	0	0	0	160	160	
	0.5	1.93	< LOQ	211	160	
	1	1.60	< LOQ	236	200	
	2	0.80	0.07	267	200	
	6	0.84	0.07	267	240	
	12	0.90	0.10	286	240	
	24	1.06	0.12	250	200	
	28	1.90	0.16	333	320	
	32	2.30	0.19	400	400	
	36	2.48	0.22	400	440	
	40	1.42	0.22	SR	SR	
Horse 3	0	0	0	148	140	
	0.5	3.39	< LOQ	200	180	
	1	3.37	< LOQ	222	220	
	2	1.79	0.16	235	200	
	6	1.78	0.17	235	200	
	12	1.97	0.20	250	200	
	24	2.45	0.29	286	240	
	28	3.93	0.32	334	300	
	32	4.78	0.36	SR	SR	
	Horse 4	0	0	0	143	120
		0.5	0.49	< LOQ	160	160
1		3.57	0.07	174	160	
2		1.39	0.12	182	160	
6		1.44	0.11	190	200	
12		1.59	0.15	190	160	
24		1.74	0.19	222	200	
28		2.03	0.23	250	200	
32		3.15	0.25	286	240	
36		2.65	0.28	286	240	
40		3.21	0.28	286	244	
44	4.36	0.25	SN	SN		
Horse 5	0	0	0	182	160	
	0.5	2.12	< LOQ	222	180	
	1	2.21	0.07	250	200	
	2	1.01	0.13	250	200	
	6	0.96	0.12	250	240	
	12	0.88	0.15	286	240	
	24	1.11	0.20	308	252	
	28	1.91	0.22	364	340	
	32	2.25	0.25	364	340	
	36	2.20	0.26	400	400	
	38	1.61	0.25	SN	SN	
Horse 6	0	0	0	174	160	
	0.5	2.61	< LOQ	222	204	
	1	3.22	< LOQ	250	216	
	2	1.23	0.08	261	240	
	6	1.14	0.08	273	256	
	12	1.37	0.11	267	272	
	24	1.41	0.14	286	296	
	28	2.35	0.18	364	320	
	32	2.73	0.18	375	328	
	36	2.75	0.18	400	332	
	40	3.02	0.20	SN	SN	
56	0.68	0.23	SR	SR		

LOQ: limit of quantification is 0.05 $\mu\text{g/ml}$ for both components. SR: sinus rhythm. SN: symptoms. During the first hour the patient received an amiodarone perfusion at a rate of 5 mg/kg/h followed for the next 23 hours at a rate of 0.83 mg/kg/h and then continued by a perfusion rate of 1.9 mg/kg/h. Horses that converted to sinus rhythm with the intravenous amiodarone treatment. Horses that did not converted with the amiodarone treatment. /: blood samples were not performed due to technical reasons.

Blood samples were taken for hematological and biochemical analysis at baseline, 1 and 4 days after termination of treatment. Blood samples for retrospective analysis of AD and desethylamiodarone (DAD) levels were taken at 0, 0.5, 1, 2, 6, 12, 24 h followed by every 4 hours until sinus rhythm or side effects appeared. These samples were analyzed by a validated high-performance liquid chromatography (HPLC) method with ultraviolet detection adapted from the method of Huy et al. (1991). All validation parameters met the criteria of the EC guidelines (Anonymous, 2002) and a limit of quantification of 0.05 µg/mL was obtained for both components. Due to technical reasons AD and DAD blood samples were not analyzed for horse 1.

Statistical analysis

The data on the effect of the treatment over time were analyzed using single-factor ANOVA. A post hoc Scheffé test was performed to detect possible significant differences among the means.

Results

All hematological and biochemical parameters were within normal limits. The results of cardiac evaluation performed before treatment by auscultation, echocardiography (2D and M-mode), Colour Flow Doppler and intra-atrial ECG are reported in Table 1. All echocardiographic measurements were within normal references except for horses 4 and 6, where the left atrial diameter in diastole was 15.66 and 15.25 cm (reference 11.3–14.52 cm) and for horses 3, 4, 5 and 6 where the left atrial diameter in systole was 14.77, 15.99, 14.96 and 15.55 cm, respectively (reference 11.22–14.48 cm)(Patteson et al., 1995).

Four out of 6 horses were successfully converted to sinus rhythm with the IV AD treatment. In three horses (1, 2 and 3), cardioversion was achieved without side effects. Horse 1 received the full treatment during 54 hours and converted 15 hours after termination of the treatment. Horses 2 and 3 converted after 40 and 32 hours of treatment, respectively. In the fourth horse (horse 6) AD treatment was discontinued after 49 hours of treatment because of the occurrence of hind limb weakness and weight shifting.

Sixteen hours after termination of the AD administration this horse converted to sinus rhythm. In horses 4 and 5, similar side effects of hind limb weakness occurred after 44 and 38 hours of treatment, respectively, and the AD infusion was terminated. These 2 horses did not convert to sinus rhythm. In all 3 animals with side effects of hind limb weakness, symptoms gradually disappeared 4–6 hours after termination of treatment.

In the first horse, AD and DAD levels were not determined. In the second and third horses, at the time of conversion, AD/DAD levels were 1.42/0.22 and 4.78/0.36 µg/mL, respectively. Upon occurrence of side-effects, AD/DAD levels in horses 4, 5 and 6 were 4.36/0.25, 1.61/0.25 and 3.66/0.27 µg/mL, respectively. The time of conversion of horse 6 was 16 hours after termination of the AD infusion. At that time AD/DAD levels were 0.68/0.23 µg/mL.

From the numerical and graphical description of the data for the AFCL and f wave intervals, it was seen that the condition of equality of variances was not suitable for statistical analyses for AFCL and f wave interval. Therefore, the ANOVA was performed on the ln-transformed data for AFCL and f waves, and revealed a highly significant difference in the mean values over time ($P < 0.001$). When compared to baseline value, the post hoc Scheffé test showed a significant difference from 2 hours for AFCL and from 24 hours for f wave interval ($0.01 < P$

< 0.05). For the other parameters studied, i.e., QT, QRS and HR, the Scheffé test did not reveal any differences between the mean measured values ($P > 0.05$).

Twenty four hours after cessation of AD treatment hematological and biochemical parameters remained within normal limits, except for total bilirubin which was slightly increased in horses 4, 5 and 6. The horses showed no clinical abnormalities and 4 days after cessation of treatment total bilirubin had returned to normal values.

One week after AD treatment horses 4 and 5 were treated with oral QS. At that time both horses showed an AFCL that was longer than at the start of the AD treatment (horse 4: 160 ms versus 143 ms; horse 5: 267 ms versus 182 ms).

After a total of 11 doses of QS, in horse 4, treatment was terminated because of adverse side effects (>25% widening of the QRS complex, tachycardia). Conversion to sinus rhythm was not achieved. Horse 5 converted successfully to sinus rhythm after two doses of QS. During the treatment this horse showed a mild abdominal discomfort.

Discussion

Amiodarone, a K⁺- channel blocker, is an efficacious and safe anti-arrhythmic drug used in humans to treat atrial fibrillation, atrial flutter and ventricular fibrillation (Anderson, 1995; Kerin et al., 1996; Kodama et al., 1997; Podrid, 1995; Singh, 1995). By inhibiting outward potassium currents, AD prolongs the repolarization which may lead to AF termination (Sugiyama et al., 2001).

The dose for the first 24 h of AD infusion was extrapolated to the weight of a horse from reported human doses (Kerin et al., 1996; Kochiadakis et al., 1999a; Kochiadakis et al., 1999b; Vardas et al., 2000). In humans, after a 24-hour infusion, treatment is usually continued orally (Canada et al., 1983; Kochiadakis et al., 1999a; Kochiadakis et al., 1999b). However, we used an IV treatment because preliminary pharmacokinetic results in horses had indicated a low bioavailability of orally administered AD (D. De Clercq unpublished data).

In the human condition, conversion occurred at a plasma AD concentration between 0.6 and 3.0 µg/mL with a mean of 2.541±1.321 µg/mL (Canada et al., 1983; Vardas et al., 2000). Tieleman et al. (1997) calculated that a conversion was unlikely to occur at plasma AD concentration less than 1.2 µg/mL. The average plasma concentration of DAD was 0.34±0.241 µg/mL (Vardas et al., 2000) and 1.4±0.7 µg/mL (Tieleman et al., 1997). However, Rotmensch et al. (1984) described a weak or even an absent concentration-effect correlation. Some studies describe that for conversion of AF concentrations of DAD was more important than those of AD (Balsler et al., 1991; Nattel et al., 1988). A delayed therapeutic effect after withdrawal of therapy is described (Latini et al., 1984).

Despite the fact that none of the horses showed significant atrioventricular valve regurgitation, left atrial dimensions were out with the normal limit in four horses (Patteson et al., 1995). This might be explained, at least in part, by the fact that references for Thoroughbreds were used while our horses were Warmbloods.

Although in horse 5 only a slightly enlarged left atrium and a long baseline AFCL were found, AD treatment failed. The fact that in the following week, only 2 doses of QS were needed to cardiovert this horse could be explained by a synergistic effect of both drugs. AD is known to have a long half-life in humans (15–47 days) (Canada et al., 1983; Chow, 1996) and ponies (Trachsel et al., 2004) and at the onset of the QS treatment the AFCL (267 ms) was substantially longer than at the baseline (182 ms) (Pandozi et al., 2003; Sager, 2000). A

longer AFCL means that less fibrillation waves can coexist in the atria and that AF termination is more likely (Wijffels et al., 1995).

Horse 4 could not be converted at all. This could be explained by the fact that this animal had the longest AF duration, the largest atrial size and also the shortest AFCL, all factors leading to an increased AF stability (van Loon et al., 2000; 2001; Vardas et al., 2000; Wijffels et al., 1995).

In human medicine, adverse effects due to chronic doses of AD are thyroid, pulmonary, hepatic, gastrointestinal, ocular and dermatological disturbances and rarely epididymitis or nerve disorders (Gill et al., 1992). High doses of AD may also slow down the sensory and motor nerve conduction velocities of the legs because of a toxic effect on the axons in the peripheral nerves (Besser et al., 1994). However, variability in blood–nerve barrier efficacy between individuals has been suggested in man (Santoro et al., 1992). These findings might explain the hind leg weakness and weight shifting observed in some of our horses. Toxic side effects with a short term AD treatment are rather rare. A few cases of acute hepatotoxicity due to IV AD administration have been reported in humans (Agozzino et al., 2002; Gonzalez et al., 2002). Three of our horses had a slightly increased total bilirubin but showed no clinical abnormalities.

The present paper describes an IV AD treatment in horses with naturally occurring chronic AF. While QS and flecainide can result in life threatening proarrhythmia (wide QRS tachycardia, torsades de pointes), the current AD protocol did not result in cardiac side effects which was considered as a major advantage. In addition non-cardiac side effects were mild and disappeared spontaneously after termination of treatment.

In order to increase success rate and decrease adverse drug effects, AD treatment regimen should be further adapted based upon pharmacokinetic and pharmacodynamic studies in horses.

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CHAPTER 5

PHARMACOKINETICS AND BIOAVAILABILITY OF INTRAVENOUSLY AND ORALLY ADMINISTERED AMIODARONE IN HORSES

EVALUATION OF THE PHARMACOKINETICS AND BIOAVAILABILITY OF INTRAVENOUSLY AND ORALLY ADMINISTERED AMIODARONE IN HORSES

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Summary

The objective of the study was to determine the clinical effects and pharmacokinetics of amiodarone after single doses of 5 mg/kg administered orally or intravenously.

In a cross over study, clinical signs and electrocardiographic variables were monitored and plasma and urine samples were collected. A liquid chromatography–mass spectrometry method was used to determine the percentage of protein binding and to measure plasma and urine concentrations of amiodarone and the active metabolite desethylamiodarone.

No adverse clinical signs were observed. After IV administration, median terminal elimination half-lives of amiodarone and desethylamiodarone were 51.1 and 75.3 hours, respectively. Clearance was 0.35 [L/h]/kg, and the apparent volume of distribution for amiodarone was 31.1 L/kg. The peak plasma desethylamiodarone concentration of 0.08 µg/mL was attained 2.7 hours after IV administration. Neither parent drug nor metabolite was detected in urine, and protein binding of amiodarone was 96%. After oral administration of amiodarone, absorption of amiodarone was slow and variable; bioavailability ranged from 6.0% to 33.7%. The peak plasma amiodarone concentration of 0.14 µg/mL was attained 7.0 hours after oral administration and the peak plasma desethylamiodarone concentration of 0.03 µg/mL was attained 8.0 hours after administration. Median elimination half-lives of amiodarone and desethylamiodarone were 24.1 and 58.6 hours, respectively.

Results indicate that the pharmacokinetic distribution of amiodarone is multicompartmental. This information is useful for determining treatment regimens for horses with arrhythmias. Amiodarone has low bioavailability after oral administration, does not undergo renal excretion, and is highly protein-bound in horses.

Introduction

Amiodarone (AD) is a benzofuran-derived drug that was developed more than 35 years ago for management of myocardial ischemia in humans (Singh, 1983). Although the antiarrhythmic and preventative effects of AD on atrial flutter and atrial fibrillation (AF) have been known since the early 1980s, the drug has been used most extensively for treatment of ventricular arrhythmias (Siddoway, 2003). Because of its potency as an antiarrhythmic drug, AD is receiving renewed attention for potential use in treatment and prevention of AF (Pollak and Shafer, 2004).

AD has high lipid solubility and is taken up extensively by tissues, resulting in a high volume of distribution and prolonged elimination half-life ($t_{1/2}$). Estimates of elimination $t_{1/2}$ vary, depending on study design and sensitivity of the analytic methods used (Gill et al., 1992; Pollak and Shafer, 2004). A model for estimating pharmacokinetic variables of AD in ponies has been described (Trachsel et al., 2004). As is true in humans, AF is one of the most common cardiac arrhythmias in horses (Reef et al., 1988; Tsikouris and Cox, 2001). The major clinical sign in horses is exercise intolerance. The prognosis for return to normal sinus rhythm is influenced by the duration for which the arrhythmia has persisted and by atrial diameter; duration of the arrhythmia for longer than 6 months or development of the arrhythmia in association with atrial dilatation is associated with a poor prognosis for successful cardioversion. Atrial dilatation can be caused by mitral valve regurgitation and can also result from AF (Reef et al., 1988; Stadler et al., 1994; van Loon et al., 2001). In many horses with AF, no underlying cardiac disease is detected and treatment is often successful. After cardioversion, horses generally return to the previous level of athletic ability (Reef et al., 1988).

The standard treatment for AF is administration of quinidine sulphate (QS), although up to 76% of treated horses develop 1 or more adverse effects, including urticaria, nasal mucosal edema, colic, diarrhea, laminitis, tachycardia, anaphylactic shock, syncope, or sudden death (Marr et al., 1995; Muir et al., 1990; Reef et al., 1988; 1995; 2002; Stadler et al., 1994). In some countries, QS has become difficult to obtain and will likely become more expensive (McGurrin et al., 2003). These factors have stimulated the search for an alternative and less toxic drug for treatment of chronic AF in horses.

Multiple antiarrhythmic drugs are used for treatment of AF in humans, some of which have already been investigated for use in horses (Boriani et al., 2004). Intravenously administered

flecainide (a class IC antiarrhythmic drug) and AD (a class III antiarrhythmic drug) have been used for treatment of horses with AF (De Clercq et al., 2006; van Loon et al., 2004). The use of flecainide in horses appeared promising for treatment of acute AF in 1 study, but it was not useful in horses with chronic AF because of elicitation of ventricular dysrhythmias (Ohmura et al., 2000; van Loon et al., 2004). In another study, 4 of 6 horses were converted to sinus rhythm after IV administration of AD. In that study, horses received 5 mg of AD/kg per hour for 1 hour followed by 0.83 mg/kg/h for 23 hours and 1.9 mg/kg/h for 30 hours. Infusion was discontinued when conversion was achieved or when adverse effects were observed (De Clercq et al., 2006). Because AD appeared to be potentially useful for treatment of chronic AF in horses, the present study was undertaken to investigate the pharmacokinetics of orally and IV administered AD in horses.

Materials and Methods

The experimental protocol was approved by the Ethics Committee of the Faculty Veterinary Medicine at Ghent University.

Study design

In a cross-over format, the first phase of the study involved IV administration of a single dose (5 mg/kg) of AD (Cordarone 150mg/3mL, Sanofi-Synthélabo) to 3 healthy Standardbred mares with mean±SD age, body weight, and height at the withers of 9.8±3.5 years, 491.7±19.8 kg, and 156.3±8.9 cm, respectively, that had not been withheld from feed. Horses received the dose of AD as a bolus in the right jugular vein over a period of 2 minutes. Three other horses received an orally administered dose (5 mg/kg) of crushed tablets by means of nasogastric intubation after being withheld from feed for 12 hours. Four hours after receiving the oral treatment, horses were given hay ad libitum. Blood was withdrawn from the left jugular vein in heparinized polyethylene tubes just before drug administration; 5, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 480, and 720 minutes after administration; and every 12 hours after that until 7 days after administration. Blood samples were centrifuged at 3,000 X g immediately after collection to obtain plasma. In 3 horses that received AD IV, total urine samples were collected 0, 1, 2, 3, 4, 6, 8, and 12 hours after treatment. Plasma and urine samples were frozen and stored at -18°C until drug assay. Clinical signs, respiratory rate, and an ECG were recorded at each blood sampling time until 6 hours after drug administration. Information collected from ECG included heart rate, PR interval, QRS wave duration, and QT interval. Immediately before and 7 days after drug administration, complete hematologic and biochemical blood analyses were performed.

In the second phase of the study, which was initiated 60 days after the first phase, the same sampling protocol was followed. Horses that had received AD IV in the first phase were treated orally and vice versa.

Plasma and urine analyses

AD and desethylamiodarone (DAD) concentrations were measured by use of a validated high-performance liquid chromatography (HPLC) method and tandem mass spectrometry. In brief, 100 µL of a solution of the internal standard tamoxifen (concentration, 10 µg/mL) was added to a 1,000-µL aliquot of plasma or urine. After mixing for 15 seconds, 25 µL of glacial acetic

acid was added to deproteinize the mixture and the tube was centrifuged at 9,500 x g for 10 minutes. Thereafter, solid-phase extraction and clean up were performed by use of strong-cation-exchange cartridges (Sopachem) that had been previously conditioned with 1 mL of methanol solution and 1 mL of water. After application of the supernatant, cartridges were sequentially washed with 1 mL of 0.1M hydrochloric acid and 1 mL of methanol. Analytes were eluted with 2 mL of a methylene chloride-isopropanol-ammonia solution (78/20/2 [vol/vol/vol]). The elution liquid was evaporated at 40°C under a gentle nitrogen stream. The dry residue was dissolved in 250 µL of 0.1% formic acid in a water-methanol solution (20/80 [vol/vol]). A 100-µL aliquot of that mixture was injected onto the liquid chromatography system combined with an ion-trap mass spectrometer operating in the electrospray positive-ionization mode (Thermo Finnigan). For chromatographic separation, a C18 column (Thermo Finnigan, 5 µm; 100 X 2.1 mm internal diameter) with a guard column of the same type (5 µm; 10 X 2.1 mm internal diameter) were used. An isocratic run of 5 minutes was performed with a mobile phase of acetonitrile (A) and 0.1% formic acid in water (B; ratio, 80A:20B [vol/vol]) at a flow rate of 0.2 mL/min. Quantification was performed by use of ion transitions with mass-over-charge ratios of 646.1 > 572.8 for amiodarone and 618.2 > 546.8 for DAD.

The analytical method was validated for linearity, within-and between-day trueness and precision, limit of quantification (LOQ), and limit of detection (LOD) according to the European Community guidelines for analytical methods (Commission Decision, 2002). Calibration curves were prepared by adding AD or DAD to pooled blank plasma and urine samples at concentrations of 0.005, 0.010, 0.025, 0.050, 0.100, 0.500, 1.000, and 0 µg/mL. The trueness (i.e., recovery or the difference between the mean detected and fortified concentrations) and within-day precision of the method were determined by use of 6 blank samples to which known quantities of drug were added at 0.010 and 0.050 µg/mL for plasma and 0.025 and 0.500 µg/mL for urine. The trueness for AD in plasma was -23.9% and -10.7% for the 0.010 and 0.050 µg/mL samples, respectively, and for DAD, values of 7.5% and -1.6% were obtained for the 0.025 and 0.500 µg/mL samples, respectively. For urine samples, values were 1.0% and -0.8% for AD and -12.3% and -0.6% for DAD at concentrations of 0.025 and 0.500 µg/mL, respectively. All values were within the acceptance range of -30% to +10% for concentrations ≤ 0.010 µg/mL and -20% to 10% for concentrations > 0.010 µg/mL (Yamaoko et al., 1978). The precision also fell within the maximum relative SD values of 21.3%, 18.6%, 11.8%, and 16.7% for concentrations of 0.010, 0.025, 0.050, and 0.500 µg/mL, respectively. The between-day trueness and precision

were determined by use of samples of plasma with a drug concentration of 0.010 µg/mL and were used for quality control during analyses of the collected samples. Those values were in the specified maximum ranges. The LOQs for plasma and urine were established by analyzing 6 blank samples to which AD and DAD (concentration, 0.005 µg/mL) had been added. The LOD was calculated by means of the criterion of a signal-to-noise ratio of 3:1. This corresponded to LODs of 0.0001 and 0.00004 µg/mL, respectively, for AD and DAD in plasma and of 0.00016 and 0.00009 µg/mL, respectively, in urine.

Protein binding was determined in plasma samples (n = 6) to which drug had been added at a concentration of 2 µg/mL and allowed to equilibrate for 30 minutes at 37°C. One milliliter of that solution was centrifuged at 9,500 x g for 10 minutes through a filter of 30,000 molecular-weight cutoff. The filtrate was analyzed similarly to plasma samples.

Pharmacokinetic data analysis

The plasma concentration-time curves for data obtained after IV administration of AD in each horse were fitted by use of a nonlinear least-squares regression-fitting program (M/W Pharm, version 3.60). The model was determined for best fit on the basis of a smaller value for the Akaike information criterion (Yamaoko et al., 1978). The plasma concentration-time curves for those data best fit the 3-compartmental model. The following equation was used to describe the concentration-time curves: $C_p = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t}$, where C_p is the plasma concentration; A, B, and C are zero-time intercepts; e is the base of natural logarithms (ln); t is time; and α , β , and γ are hybrid constants dependent on first-order rate constants. Values for $AUC_{0-\infty}$, Cl, apparent Vd, and $t_{1/2el}$ were calculated according to standard pharmacokinetic equations (Gibaldi and Perrier, 1975).

For AD data obtained after oral administration and DAD data obtained after IV and oral administration (of AD), noncompartmental methods were used because standard fitting procedures resulted in poor correlations. The $AUC_{0-\infty}$ value was calculated via the trapezoidal method. The variables C_{max} and T_{max} were observed directly from the plasma concentration time plots. Absolute bioavailability (F) was calculated from the following equation:

$$F = (AUC_{0-\infty} \text{ oral} / AUC_{0-\infty} \text{ IV}) \times 100$$

Statistical analysis

Pharmacokinetic variables were reported as median values except for $t_{1/2el}$, for which a harmonic mean was calculated. Respiratory rate, heart rate, P-R interval, QRS duration, and Q-T interval over time were analyzed by use of single-factor ANOVA. The mean measured values were compared with values obtained before treatment. For all comparisons, values of $p < 0.05$ were considered significant.

Results

Administration of AD via IV and oral routes was tolerated well by all horses. Values for hematologic and serum biochemical variables remained within reference ranges for the first (i.e., immediately before administration) and second (i.e., 7 days after administration) blood samples (Carlson, 2002). Numeric and graphic descriptions of data for respiratory rate, heart rate, PR interval, QRS duration, and QT interval indicated that the condition of equality of variances was satisfied. Results of single-factor ANOVA did not reveal significant differences between mean values for the variables. Although increased heart rate was observed after IV administration of AD, the change was not significant. Pharmacokinetic variables for AD and DAD were given as median and range values and summarized (Tables 1 and 2). Mean \pm SD plasma concentrations of AD and DAD after IV and oral administration were plotted (Figures 1 and 2). In horses in the IV administration group, plasma concentrations of AD and DAD were quantifiable from 5 and 15 minutes, respectively, after administration until 168 hours after administration. In the oral administration group, AD and DAD concentrations were quantified in plasma from 30 and 90 minutes, respectively, after administration until 96 and 120 hours after administration. After IV administration, plasma concentrations of AD decreased rapidly in the first phase of the 3-compartment model. The second phase was characterized by a slower decline in concentration and was followed by a very slow decline in concentration in the third phase. In 2 horses, there was a small increase (50 and 100 μ g/mL) in plasma AD concentration at 8 and 12 hours, respectively (Figure 3). The plasma concentration curves after oral administration of the drug were variable, and absorption was slow and incomplete. Protein binding of AD as analyzed at 2 μ g/mL was 96%. No AD or DAD could be detected in the urine samples collected until 12 hours after IV administration.

Table 1. Median and range values of pharmacokinetic variables after a single IV or orally administered dose (5 mg/kg) of amiodarone (AD) in 6 healthy horses.

	Pharmacokinetic parameters of AD after 5 mg AD/kg bodyweight IV		Pharmacokinetic parameters of AD after 5 mg AD/kg bodyweight PO	
	median	range	median	range
C _{max} (µg/ml)	NA	NA	0.14	0.04 - 0.17
T _{max} (h)	NA	NA	7.0	3.0 - 12.0
AUC (µg.h/ml)	14.5	11.0 - 19.6	2.5	1.2 - 5.1
Cl ([L/h]/kg)	0.35	0.27 - 0.45	NA	NA
V _d (L/kg)	31.1	14.9 - 64.5	NA	NA
T _{1/2el} (h)	51.1*	38.2 - 84.0	24.1*	18.7 - 36.1
F (%)	NA	NA	19.4	6.0 - 33.7

IV: intravenous. PO: per oral. C_{max}: maximum concentration obtained after bolus. T_{max}: time to reach C_{max}. AUC: area under the curve. Cl: clearance. V_d: volume of distribution. T_{1/2el}: terminal elimination half-life. F: absolute bioavailability. NA: not applicable. * harmonic mean.

Table 2. Median and range values of pharmacokinetic variables for desethylamiodarone (DAD) after a single IV or orally administered dose (5 mg/kg) of amiodarone (AD) in the same 6 horses as in Table 1.

	Pharmacokinetic parameters of DAD after 5 mg AD/kg bodyweight IV		Pharmacokinetic parameters of DAD after 5 mg AD/kg bodyweight PO	
	median	range	median	range
C _{max} (µg/ml)	0.08	0.05 - 0.11	0.03	0.02 - 0.06
T _{max} (h)	1.5	0.7 - 8.0	8.0	6.0 - 14.0
AUC (µg.h/ml)	7.3	4.2 - 10.3	2.1	1.2 - 6.7
T _{1/2el} (h)	75.3*	53.8 - 127.1	58.6*	48.0 - 100.8

IV: intravenous. PO: per oral. C_{max}: maximum concentration obtained after bolus. T_{max}: time to reach C_{max}. AUC: area under the curve. T_{1/2el}: terminal elimination half-life. * harmonic mean.

Figure 1. Mean \pm SD plasma concentrations of amiodarone and desethylamiodarone after a single IV administered dose (5 mg of amiodarone/kg) in 6 healthy horses. The concentration axis is expressed in a semi-logarithmic scale.

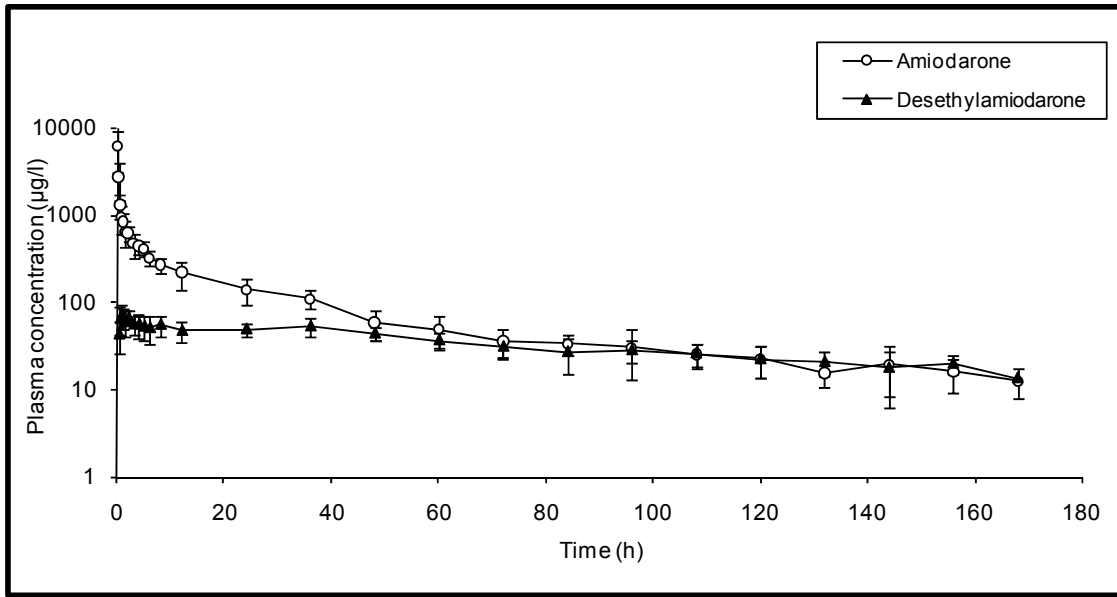
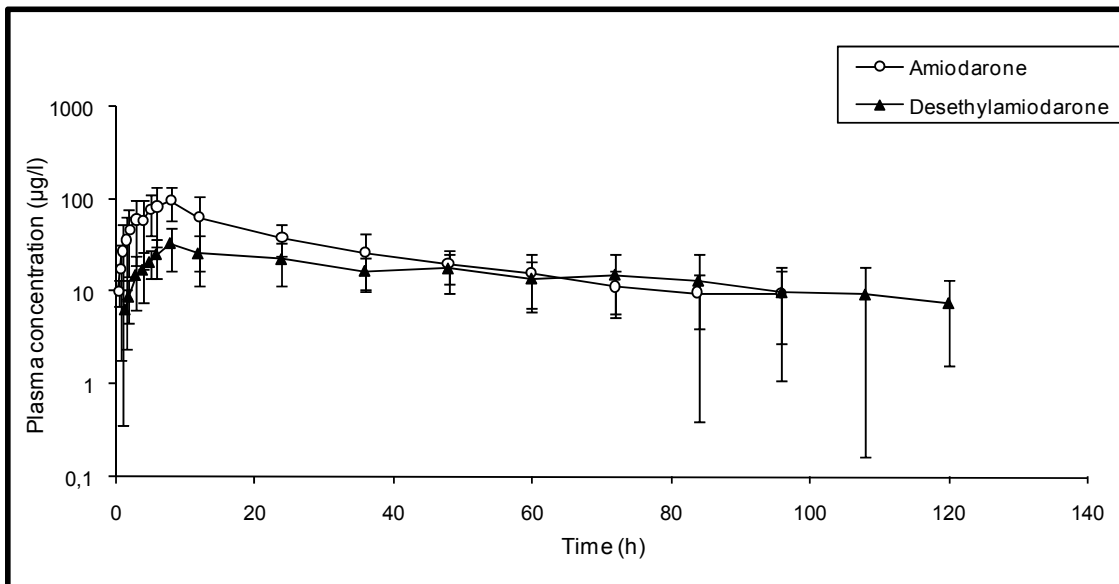


Figure 2. Mean \pm SD plasma concentrations of amiodarone and desethylamiodarone after a single orally administered dose (5 mg/kg) of amiodarone in the same horses as Figure 1. The concentration axis is expressed in a semi-logarithmic scale.



Discussion

The dose (5 mg/kg) of AD used in the present study was chosen on the basis of doses described in pharmacokinetic studies in other species. In this study, there were no changes in respiratory rate, P-R interval, QRS duration, or QT interval and no arrhythmias developed after the single IV dose of AD, findings that corroborated with those from a study in humans (Chow, 1996). Substantial prolongation of the QT interval without torsades de pointes, however, has been described after administration of several doses of AD in humans and dogs and has also been reported after a single IV dose in ponies (Hohnloser et al., 1991; van Opstal et al., 2001; Trachsel et al., 2004). Horses in the present study had a moderate increase in heart rate after IV administration of AD, a finding that has also been reported in ponies and humans; in contrast, a decrease in heart rate after a single dose of AD has been described in dogs (Connolly et al., 1984; Trachsel et al., 2004; Wellens et al., 1984). As hypothesized in human medicine, the higher heart rate may be explained by the development of hypotension after IV administration (Gill et al., 1992; Vardas et al., 2000; Wellens et al., 1984).

Pharmacokinetic variables reported for AD vary. This is likely a result of the length of time during which samples were collected in the experiments and the sensitivity of the assays (Pollak et al., 2000; Weiss et al., 1999). In the present study, bolus-dose administration and sampling times of 7 days were used to determine bioavailability and calculate standard 2-stage compartmental pharmacokinetic variables. The method of analysis was a liquid chromatography–tandem mass spectrometry method performed on an ion-trap mass spectrometer. These methods yielded an LOQ of 0.005 µg/mL in the plasma and urine samples. Other investigators obtained lower LOQ values by use of either another tandem mass spectrometer system with a more sensitive triple-quadrupole instrument or techniques involving the use of radiolabeled AD (Meng et al., 2001). With the latter methods, the terminal elimination phase can be better characterized and a better estimate of elimination $t_{1/2}$ can be obtained. In humans studies, investigators described a terminal elimination phase of 100 days and an elimination $t_{1/2}$ of 58 days after a single dose of AD (Meng et al., 2001; Pollak et al., 2000), findings that were in accordance with values reported after long-term administration or use of radiolabeled drug (Broekhuijzen et al., 1969). A drawback in methods involving the use of radiolabeled drug is that it is not always possible to differentiate between parent drug and metabolites. Comparison of pharmacokinetic variables should, therefore, be performed with data collected under the same circumstances, including similar sampling times and sensitivity of analytic methods.

The concentration-versus-time curves for data obtained after IV administration were fitted to a 3-compartment model. Values for mean Vd (31.1 L/kg), Cl (0.35 [L/h]/kg), and $t_{1/2}$ (38 to 84 hours) were calculated. Compared with our value, a much longer $t_{1/2}$ of 16.3 days was calculated in ponies treated with AD by another group (Trachsel et al., 2004). This difference may be explained because a mathematical based pharmacokinetic model was used to estimate pharmacokinetic parameters in that study.

As has been reported in studies in humans, the plasma concentration-time curves in these horses after oral administration did not have a good fit for the curve on the basis of compartmental modeling (Andreasen et al., 1981; Haffajee et al., 1983). A delay (0.5 hours) in the appearance of AD in plasma was observed in these horses. In humans, mean lag times of 0.5 to 1.4 hours have been reported (Canada et al., 1983, Haffajee et al., 1983; Plomp et al., 1984). Maximum plasma AD concentrations were attained 7.0 hours after administration in our horses, whereas mean \pm SD values of 7.3 \pm 2.9 hours, 5.2 \pm 0.6 hours and 4.8 \pm 2 hours have been reported in humans (Andreasen et al., 1981; Canada et al., 1983, Haffajee et al., 1983). A lower median C_{max} (0.14 μ g/mL) was observed in our horses, compared with values of 0.5 μ g/mL, 1.7 μ g/mL, and 0.37 \pm 0.22 μ g/mL reported in humans (Andreasen et al., 1981; Haffajee et al., 1983; Plomp et al., 1984). Median bioavailability in the nonfed horses (19.4%) was < 50%, a value that has been reported in dogs and rats, whereas in humans, values of 22% to 86% have been reported (Andreasen et al., 1981; Broekhuizen et al., 1969; Canada et al., 1983; Holt et al., 1983; Plomp et al., 1984; Riva et al., 1982). The large variability in absorption observed in the present study was in agreement with findings from pharmacokinetic studies in humans. Such low and variable bioavailability may be attributed to incomplete absorption of the drug and first-pass metabolism in the intestine or liver (Plomp et al., 1984). Concurrent ingestion of food has a positive influence on bioavailability of AD in humans; investigators in that study concluded that AD has dissolution–rate-limited absorption and the effects of food and bile result in the improved bioavailability (Meng et al., 2001). Whether food intake also increases bioavailability of orally administered AD in horses remains to be investigated.

A secondary peak in plasma concentration 8 to 12 hours after IV administration was observed in 2 horses and may have been a result of entero-hepatic cycling (Andreasen et al., 1981). The fact that the highest bioavailability for AD was observed in those 2 horses supports this theory.

Plasma concentrations of DAD were measured simultaneously in each sample. This metabolite is important because it has pharmacologic potency and toxicity equal to the parent drug (Gill et al., 1992). After administration of the drug in our study, median maximum DAD concentrations were 80 and 29 $\mu\text{g/L}$ after IV and oral administration, respectively. The $t_{1/2}$ of DAD was longer than the $t_{1/2}$ of amiodarone, a finding that was in accordance with data in humans (Plomp et al., 1984). In an earlier study in which AD was given as an IV infusion, plasma DAD concentrations remained low and did not exceed 360 $\mu\text{g/L}$, a fraction of the amiodarone concentration (De Clercq et al., 2006). This observation has also been made in rats (Wyss et al., 1990). These findings are different from those reported in a study in which chronic AD administration in humans yielded plasma DAD concentrations nearly equal to AD concentrations (Pollak et al., 2000). This may indicate that there is a species-dependent difference in metabolism, with N-dealkylation being a less important metabolic pathway in horses than in humans. However, long-term administration studies should be performed, with analysis of plasma and liver tissue for AD and DAD concentrations, and in vitro experiments with microsomes obtained from equine liver tissue could be performed to confirm this hypothesis.

We observed 96% protein binding for AD in the in vitro ultrafiltration experiments. The protein binding was investigated at 2 $\mu\text{g/mL}$ since that concentration appears to be a pharmacologically important plasma concentration (Canada et al., 1983; Vardas et al., 2000). Some investigators have questioned the reliability of the ultrafiltration technique for quantification of AD protein binding because of adherence of drug molecules to the filtration devices, but those experiments were conducted with radiolabeled AD; other researchers reported no interference when using analytic-grade AD (Ujhelyi et al., 1996; Veronese et al., 1988). The high protein binding we observed was in agreement with findings in other species (Andreasen et al., 1981; Latini et al., 1984; Veronese et al., 1988).

No AD or DAD was detected in urine of horses during the 12 hours following IV administration. Because glucuronide conjugates have been detected in urine of treated humans, urine samples in the present study were combined with sodium hydroxide to deglucuronidate the samples, but neither AD nor DAD was detected. Similar results have been described in humans and rats (Haffajee et al., 1983; Latini et al., 1984; Plomp et al., 1984; Wyss et al., 1990). In a study involving rats, little unchanged AD or DAD was eliminated in feces or urine (Wyss et al., 1990). In that study, 94% and 1.7% of a dose of radiolabeled drug was eliminated in feces and urine, respectively, primarily as other

nonspecified radioactive metabolites formed from AD and DAD (Wyss et al., 1990). This finding is in contrast to those predicted by the mathematical model used in an earlier study, in which 96% of AD was eliminated as DAD in the urine of ponies (Trachsel et al., 2004). These results indicate that metabolism of AD into other compounds is the primary form of elimination. The metabolic fates of AD and DAD are unclear. The metabolism of AD and DAD in several species, including humans, rats, and rabbits, has been reviewed (Ha and Follath, 2004). AD is dealkylated to DAD in the liver and intestine, primarily in hepatic microsomes. A hydroxylation step in the metabolism of AD has been proposed (Ha and Follath, 2004). DAD may be hydroxylated, dealkylated, and deaminated. Involvement of cytochrome P450 enzymes and species-dependent metabolic steps have been proposed (Ha and Follath, 2004). Deiodination of AD has also been observed, 1 result of which is excretion of free iodide in the urine (Rao et al., 1986).

The toxicity of AD has already been known for a long period of time. In the early 1980s, physicians were reluctant to prescribe AD because of adverse effects associated with chronic administration (Pollak et al., 2000). The uncommon pharmacokinetic features of AD and DAD (i.e., slow filling of the third compartment of the 3-compartment model and a gradual increase in plasma concentrations toward steady state) are partly responsible for the reluctance to administer AD. In this study, no clinically important adverse effects or changes in results of serum biochemical analyses were observed. In human medicine, acute adverse effects associated with IV administration of AD are rare. Hypotension is the most common reaction and develops as a result of the vasodilatory and negative inotropic effects of AD. The vasodilatory effect is caused by the solvent (polysorbate 80) in which the drug is suspended and can be managed with dose reduction or routine pressure-supportive measures (Chow, 1996). Development of acute hepatotoxicosis after IV administration of AD has been reported in humans (Agozzino et al., 2002; Gonzalez et al., 2002).

Adverse effects associated with chronic (eg, months to years) administration of AD are more frequent and involve the lungs, liver, heart, thyroid gland, gastrointestinal tract, eyes, skin, nerves, and, rarely, the epididymis (Gill et al., 1992). Gastrointestinal adverse effects in humans include nausea, anorexia, and constipation. Neurologic adverse effects include ataxia, paresthesia, and development of tremors. In general, clinical signs of toxicosis are dose-related and disappear after withdrawal of treatment. Guidelines for treating adverse reactions have been described (Siddoway, 2003).

The purpose of the present study was to determine the pharmacokinetics of AD in horses to facilitate development of treatment protocols for horses with acute-onset or chronic AF. An oral treatment protocol could be developed, but associated disadvantages include drug bioavailability that is low and variable among individuals and the long delay between initiation of treatment and onset of suppression of the arrhythmia. Therefore, several weeks may be required to achieve steady-state plasma concentrations and drug costs may be prohibitive. Moreover, failure of long-term AD treatment (65 mg/kg, q 24 hours for 30 days) to resolve AF has been reported in 2 horses (Guglielmini et al., 2003).

AD may be more useful if administered according to a continuous IV infusion protocol. This method of administration has the advantage of rapid filling of the deep compartments and possibly a more rapid onset of action that could lead to cardioversion. Disadvantages associated with this treatment method are that the treatment must be administered in a clinical setting and that, depending on the dose, a higher risk of adverse effects may exist. One protocol for IV infusion of AD has been published; mild signs of hind limb weakness and weight shifting were reported (De Clercq et al., 2006). A serial IV dosing schedule, as was proposed by 1 group of investigators, would be easier to implement in practice, but no clinical data from horses treated via this protocol have been published (Trachsel et al., 2004). Another possibility would be a treatment protocol combining IV and oral dosing, in which the IV dose could be administered in a clinic setting and plasma drug concentrations could be measured. Use of such a protocol would potentially permit slower increases in plasma drug concentrations toward the desired steady-state concentration with fewer adverse effects but would have the disadvantage of increased treatment costs.

Results of the present study confirm that the pharmacokinetics of AD and DAD in horses are multicompartmental. The drug is poorly bioavailable after oral administration, does not undergo renal excretion, and is highly protein-bound, similar to findings in other species. However, further pharmacokinetic and pharmacodynamic studies are needed to develop a safe treatment protocol for AD in horses. Studies of long-term dosing and clinical effects and use of more sensitive analytic techniques are needed before AD can feasibly be administered to horses with chronic AF.

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CHAPTER 6

ADAPTED AMIODARONE TREATMENT PROTOCOL IN HORSES WITH ATRIAL FIBRILLATION

EFFECTS OF AN ADAPTED INTRAVENOUS AMIODARONE TREATMENT PROTOCOL IN HORSES WITH ATRIAL FIBRILLATION

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Summary

Good results have been obtained with a human amiodarone (AD) IV protocol in horses with chronic atrial fibrillation (AF). A pharmacokinetic study of AD in horses revealed an adapted IV AD treatment protocol for horses.

Six horses with chronic AF were treated with an adapted AD infusion protocol. The protocol consisted of 2 phases with a loading dose followed by a maintenance infusion. In the first phase, horses received an infusion of 6.52 mg AD/kg/h for 1 hour followed by 1.1 mg/kg/h for 47 hours. In the second phase, horses received a second loading dose of 3.74 mg AD/kg/h for 1 hour followed by 1.31 mg/kg/h for 47 hours. Clinical signs were monitored, a surface ECG and an intra-atrial electrogram were recorded. AD treatment was discontinued when conversion or any side effects were observed.

Three of the six horses cardioverted successfully without side effects. The other three horses did not convert and showed adverse effects, including diarrhea. In the latter, there were no important circulatory problems, but the diarrhea continued for 10-14 days. The third horse had to be subjected to euthanasia because a concomitant Salmonella infection worsened the clinical signs.

The applied treatment protocol based upon pharmacokinetic data achieved clinically relevant concentrations of AD and desethylamiodarone. Intravenous AD has the potential to be an alternative pharmacological treatment for AF in horses, although AD may lead to adverse drug effects, particularly with cumulative dosing.

Introduction

Atrial fibrillation (AF) is the most common pathological cardiac arrhythmia in horses. Some horses may have a history of exercise intolerance, exercise-induced epistaxis, respiratory disease, weakness or syncope. In pleasure horses, it may be diagnosed by coincidence during clinical examination for a completely different purpose (Deem and Fregin, 1982; Reef and McGuirk, 2002).

AF often occurs in horses without underlying macroscopic cardiac pathology (Deem and Fregin, 1982; Reef et al., 1988). These animals have a good prognosis for cardioversion and generally return to their previous level of athletic performance after successful treatment. However, when AF persists for >3–6 months or when atrial dilatation is present because of mitral valve regurgitation or AF *per se*, treatment becomes more difficult and AF recurrence is more common (Reef et al., 1988; van Loon et al., 2001).

For many years, quinidine sulphate (QS) has been used as the standard treatment for AF and has an efficacy of 85% in horses with AF of duration <2 months and without the presence of a detectable cardiac disease (Deem and Fregin, 1982; Reef et al., 1988). Nevertheless, in up to 76% of treated horses this anti-arrhythmic drug can yield serious cardiac and noncardiac side effects including urticaria, nasal edema, colic, diarrhea, laminitis, polymorphic ventricular tachycardia, anaphylactic shock, syncope or sudden death (Reef et al., 1995, 1988).

Because QS has become more difficult to obtain and is expensive, alternative pharmacological anti-arrhythmic treatments for AF in horses have been investigated recently (McGurrin et al., 2003). After encouraging results in experimental, acute AF in horses (Ohmura et al., 2000), flecainide, a class I anti-arrhythmic drug, showed little potential in clinical cases, and was associated with important ventricular pro-arrhythmia (van Loon et al., 2004).

Recently, the use of amiodarone (AD) IV, a class III anti-arrhythmic drug, has been described in horses suffering from chronic AF (De Clercq et al., 2006a). Horses received 5 mg AD/kg/h for 1 hour followed by 0.83 mg/kg/h for 23 hours and, subsequently, 1.9 mg/kg/h for 30 hours. With this protocol, four out of six horses successfully converted into sinus rhythm. However, three of the six horses showed adverse effects of hindlimb weakness that disappeared completely within 4–6 hours after termination of the IV AD treatment. One week after the AD treatment, the 2 horses that had not converted to sinus rhythm were treated with

QS per os. One horse successfully converted to sinus rhythm but, in the other, QS treatment had to be terminated because of adverse side effects (>25% widening of the QRS complex, tachycardia). Subsequently, this horse was used as a broodmare.

The current study was undertaken with the aim of assessing whether a pharmacokinetic-based treatment protocol of IV AD in horses with chronic AF had improved efficacy and decreased adverse drug rate. In these protocols, a loading dose and a maintenance dose was used to achieve a rapid steady state concentration. When using only a maintenance dose, the time to reach 90% of the steady state concentration was 3.32 half-lives (Shargel and Yu 1993) or, with the pharmacokinetic data of AD used in this study, about 40 hours.

This report describes the results of a pharmacokinetic-based treatment protocol of IV AD in horses with chronic AF.

Materials and methods

Cases

Five Warmblood horses and one Standardbred (horse 6) were referred for exercise intolerance (horses 1, 5 and 6), epistaxis (horses 1, 2, 3 and 5) or arrhythmia (horses 4 and 6) and diagnosed with AF. Mean±SD age, bodyweight and height at the withers were 10.3±4.4 years (2–15 years), 582.0±93.4 kg (436–720 kg) and 168.3±11.7 cm (155–190 cm), respectively. All horses were used for trotting, jumping or pleasure riding. Based upon history and clinical examination, AF duration was estimated to be between 1 and 8 months. The clinical examination, hematology and full cardiac evaluation were within normal references. Echocardiography included auscultation, surface ECG, 2-dimensional, M-mode and Colour Flow Doppler. The 2-dimensional and M-mode measurements from the left cardiac window included left atrial diameter during ventricular diastole and systole and, from the right cardiac window, aortic diameter in diastole, left ventricular internal diameter in diastole and systole, interventricular septum thickness in diastole and systole and left ventricular fractional shortening (Patteson et al. 1995) (Table 1).

Two weeks previously, horse 6 had converted with 9 doses of QS (22 mg/kg), but relapsed in AF after one week.

Adapted treatment regimen based on pharmacokinetic parameters

Based on the results of a previous study, where a steady state concentration of 1.0 µg/ml was found (De Clercq et al., 2006a) and the literature concerning the therapeutic window of AD (Haffajee et al., 1983), a decision was made to aim for a steady state of 2.0 µg/ml of AD in a first phase of 2 days and a steady state of 2.5 µg/ml of AD in the second phase of 2 days. This adjustment was based on the pharmacokinetic parameters that were found after a bolus administration of 5 mg/kg (De Clercq et al., 2006b) in test horses. The parameters used were obtained after analysis of a 2-compartment model until the 24-hour point. The following formula was used to calculate the loading and maintenance doses to obtain a steady state concentration of 2.0 and 2.5 µg/ml in these horses (Shargel and Yu, 1993):

$$C_p = \frac{R}{V_p k} \left[1 - \left(\frac{k-b}{a-b} \right) e^{-at} - \left(\frac{a-k}{a-b} \right) e^{-bt} \right]$$

In this formula, C_p is the plasma concentration, R is the rate of infusion, V_p is the volume of distribution of the central compartment, k is the elimination rate constant, a and b are hybrid constants and t is time. These individual results were averaged and used in the following protocols:

In the first phase, horses received a loading dose of 6.52 mg AD/kg/h IV (Cordarone 150 mg/3 mL, Sanofi-Synthélabo) for 1 hour, followed by 1.1 mg/kg/h IV for 47 hours. In the second phase, horses received a second loading dose of 3.74 mg of AD/kg/h IV for 1 hour followed by 1.31 mg/kg/h for 47 hours. Infusion was discontinued if conversion to sinus rhythm was achieved or side effects observed.

Recorded data

Before treatment and at regular time intervals, clinical signs and surface ECG were monitored. From the latter, heart rate (HR), mean interval between f waves (ms), QRS duration (ms) and QT interval (ms) were measured. The atrial fibrillation cycle length (AFCL) was recorded from an intra-atrial electrogram. With a temporary pacing catheter placed in the right atrium, the local atrial electrogram was recorded using a pacemaker (Thera D, Medtronic) and a pacemaker programmer (Programmer 9790, Medtronic). From this electrogram the AFCL, the time between 2 successive atrial depolarization waves, was recorded (van Loon et al., 1998). The mean interval between the f waves and AFCL were estimated from 2-10 s.

Before and at regular time intervals during treatment, plasma was collected and stored at -18°C for later AD and desethylamiodarone (DAD) analysis. Plasma was collected just before AD administration and at 0.5, 1, 2, 4, 6, 12, 24 and 36 hours (first phase) and at 48, 48.5, 49, 50, 54, 60, 72 hours (second phase) or until sinus rhythm or adverse effects appeared. After termination of the infusion protocol, plasma was collected daily for 5 days for AD and DAD plasma concentrations.

Plasma levels were analyzed by a validated high performance liquid chromatography combined with UV detection (Maes et al. 2006). A limit of quantification of 0.05 µg/ml was obtained for both components.

Haematological and biochemical blood analysis were performed before drug administration and at 24 hours and 7 days after cessation of treatment.

Statistical analysis

The effect of the treatment over time on electrocardiographic variables was analyzed using single-factor ANOVA. A Dunnet's test was performed to detect possible significant differences among baseline values.

Results

The results of cardiac evaluation by auscultation, echocardiography (2D and M-mode) and Colour Flow Doppler are presented in Table 1. The f-wave interval, AFCL, AD and DAD plasma concentrations during treatment are shown in Table 2, Figures 1 and 2.

Three of the 6 horses were converted successfully to sinus rhythm without side effects at 24 hours (Horses 2 and 3) and at 46 hours (Horse 1). In the other 3 horses, AD treatment was discontinued because of the occurrence of diarrhea with clinical signs of depression and poor appetite during 48 (horse 5), 54 (horse 4) and 72 hours (horse 6) of AD treatment. None of these 3 horses converted to sinus rhythm and they were treated with fluid administration, antimicrobial drugs IV, flunixin meglumine IV (1.1 mg/kg), vitamin E per os (6000 iu) and fecal transfaunations for 10-14 days. Clinical signs disappeared gradually in horses 4 and 5 within 10 and 14 days, respectively; 3 fecal samples for culture did not reveal Salmonella or Clostridium infection. However, 2 days after termination of AD treatment, horse 6's general condition suddenly deteriorated. This animal showed severe clinical signs of anorexia, toxemia, dehydration and laminitis because of a concomitant Salmonella infection. Despite intensive treatment, the horse had to be subjected to euthanasia 10 days after AD treatment.

After 24 hours, the mean \pm SD AD plasma level was 2.01 \pm 0.33 μ g/mL (n = 6). At the end of the first phase, the mean \pm SD AD level was 2.64 \pm 0.58 μ g/mL (n = 4). Two out of 6 horses reached the second phase and at the end of infusion, the concentrations were 2.51 and 2.57 μ g/mL. At the time of conversion, AD/DAD plasma concentrations were 2.02/0.38 μ g/mL, 2.17/0.16 μ g/mL and 1.77/0.15 μ g/mL in horses 1, 2 and 3, respectively. At the time of the occurrence of side effects AD/DAD plasma concentrations were 2.51/0.34, 3.43/0.26 and 2.57/0.45 μ g/mL in horses 4, 5 and 6, respectively.

Plasma AD and DAD concentrations decreased gradually after cessation of the treatment. Five days after termination, AD/DAD plasma concentrations were 0.23/0.30 μ g/mL (horse 1), 0.20/0.12 μ g/mL (horse 2), 0.15/0.12 μ g/mL (horse 3), 0.21/0.25 μ g/mL (horse 4), 0.43/0.12 μ g/mL (horse 5) and 0.90/0.40 μ g/mL (horse 6).

From the numerical description of the data for AFCL, f wave interval, QRS, QT and HR as well as from the error bar graphs, it was concluded that the condition of equality of variances was satisfied. The ANOVA revealed a highly significant difference in the mean values over time for AFCL and f wave interval ($p < 0.001$). When compared to baseline value, the

Dunnett's test showed a significant increase in difference for both parameters from 1 h and further ($p < 0.05$). For QT interval, QRS duration and HR, the Dunnett's test did not reveal any differences over time between the mean measured values ($p > 0.05$).

Haematological and biochemical parameters remained within normal limits 24 h after cessation of AD treatment, except that the serum total bilirubin concentration was slightly increased in horses 4 and 6. Seven days after cessation of treatment, the serum total bilirubin had returned to within the reference range in horse 4 but remained slightly increased in horse 6.

Table 1. Clinical characteristics of the study population (6 horses).

	Horse 1	Horse 2	Horse 3	Horse 4	Horse 5	Horse 6
General information						
Age (years)	11	15	10	12	12	2
Height at the withers (cm)	190	165	170	164	166	155
Weight (kg)	720	608	616	526	550	436
Duration of AF (months)	5	2	1	8	3	?
Cardiac examination						
Auscultation	2/6 Holosystolic over MV	1/6 Holosystolic over MV	No murmurs	No murmurs	No murmurs	2/6 Holosystolic over MV and 4/6 holosystolic over TV
Echographic examination						
<i>Echocardiography (2D and M-mode)</i>						
LADd (cm)	14.5	13.3	12.8	13.6	11.4	11.7
LADs (cm)	14.5	13.7	13.4	13.9	12.4	12.7
Ao (cm)	8.1	7.2	7.1	7.2	7.0	7.0
LVIDd (cm)	12.0	11.6	12.0	11.3	11.1	12.3
LVIDs (cm)	7.7	7.9	8.3	7.1	6.8	7.5
FS (%)	35.6	31.7	30.8	36.7	39.2	39.4
<i>Colour flow Doppler</i>						
Mitral valve	trivial R	trivial R	normal	normal	normal	trivial R
Tricuspid valve	normal	normal	normal	normal	normal	moderate R

AF: atrial fibrillation. LADd: left atrial diameter during ventricle diastole. LADs: left atrial diameter during ventricle systole. Ao: aortic diameter in diastole. LVIDd: left ventricular internal diameter in diastole. LVIDs: left ventricular internal diameter in systole. FS: left ventricular fractional shortening. TV: tricuspid valve area. MV: mitral valve area. R: regurgitation.

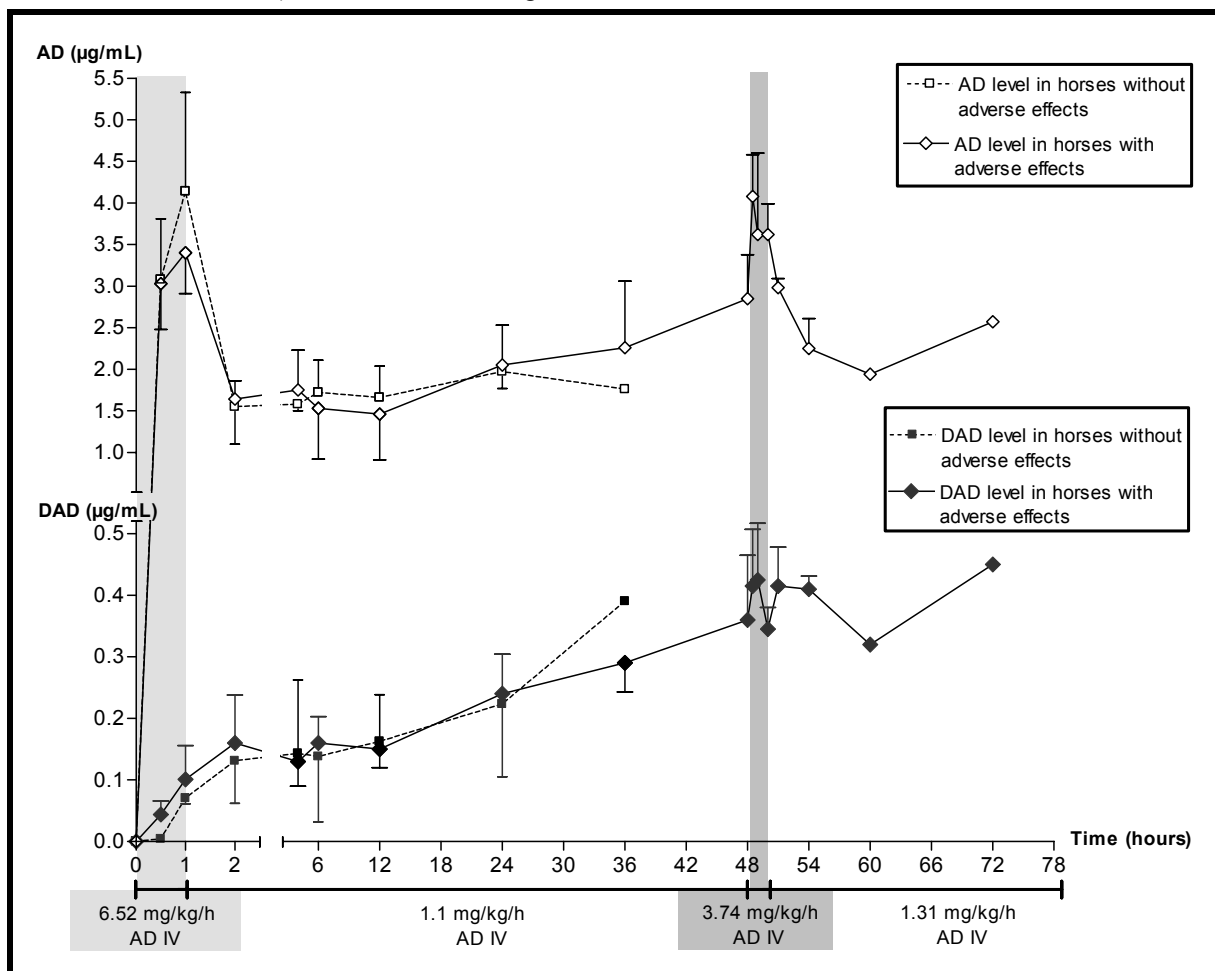
Table 2. Amiodarone (AD) and desethylamiodarone (DAD) plasma concentrations, f wave interval and atrial fibrillation cycle length (AFCL) for each horse.

	Time (hours)	AD (µg/ml)	DAD (µg/ml)	AFCL (ms)	f wave
					interval (ms)
Horse 1	0	0	0	190	160
	0.5	3.85	< LOQ	266	240
	1	4.29	0.08	285	240
	2	1.46	0.21	285	240
	4	1.67	0.28	307	260
	6	1.44	0.26	307	280
	12	1.25	0.25	364	300
	24	1.98	0.36	364	300
	36	1.76	0.39	400	320
46	2.02	0.38	SR	SR	
Horse 2	0	0	0	167	160
	0.5	2.99	< LOQ	200	180
	1	5.25	0.07	235	208
	2	2.04	0.08	250	240
	4	1.52	0.06	250	240
	6	1.56	0.06	250	248
	12	1.99	0.11	333	260
	24	2.17	0.16	SR	SR
Horse 3	0	0	0	167	160
	0.5	2.4	< LOQ	250	180
	1	2.88	0.06	267	220
	2	1.16	0.10	308	220
	4	1.56	0.09	286	260
	6	2.18	0.09	286	260
	12	1.74	0.13	286	260
	24	1.77	0.15	SR	SR
	Horse 4	0	0	0	160
0.5		2.46	0.05	211	160
1		2.91	0.09	250	220
2		1.82	0.21	250	220
4		2.11	0.17	250	220
6		1.51	0.18	267	260
12		1.12	0.19	267	260
24		1.73	0.29	267	240
36		1.58	0.31	286	240
48		2.96	0.47	308	300
48.5		4.44	0.48	308	300
49		4.31	0.49	333	300
50		2.59	0.37	333	300
54	2.51	0.34	AE	AE	
Horse 5	0	0	0	190	180
	0.5	3.55	< LOQ	267	240
	1	3.89	0.05	286	260
	2	1.70	0.07	286	260
	4	1.93	0.09	308	260
	6	2.15	0.11	286	260
	12	2.13	0.12	364	280
	24	2.6	0.17	364	280
	36	3.14	0.24	364	280
48	3.43	0.26	AE	AE	
Horse 6	0	0	0	133	120
	0.5	3.08	0.06	235	180
	1	3.39	0.16	267	240
	2	1.4	0.20	267	240
	4	1.2	0.13	267	280
	6	0.94	0.19	364	320
	12	1.14	0.15	364	320
	24	1.81	0.27	364	320
	36	2.06	0.33	364	340
	48	2.15	0.36	364	340
	48.5	3.73	0.35	364	340
	49	2.93	0.36	400	360
	50	2.06	0.32	400	360
54	2.00	0.37	400	360	
60	1.94	0.32	400	360	
72	2.57	0.45	AE	AE	

LOQ: limit of quantification is 0.05 µg/ml for both components. SR: sinus rhythm. AE: adverse effects. During the first phase an intravenous amiodarone loading dose was administered at a rate of 6.52 mg/kg/h (□) for 1 hour followed by an infusion at a rate of 1.1 mg/kg/h for the next 47 hours. In the second phase a second loading dose at a rate of 3.74 mg/kg/h (■) for 1 hour (from hour 48 to hour 49) was followed by an infusion at a rate of 1.31 mg/kg/h. □ Horses that converted into sinus rhythm with the intravenous amiodarone treatment.

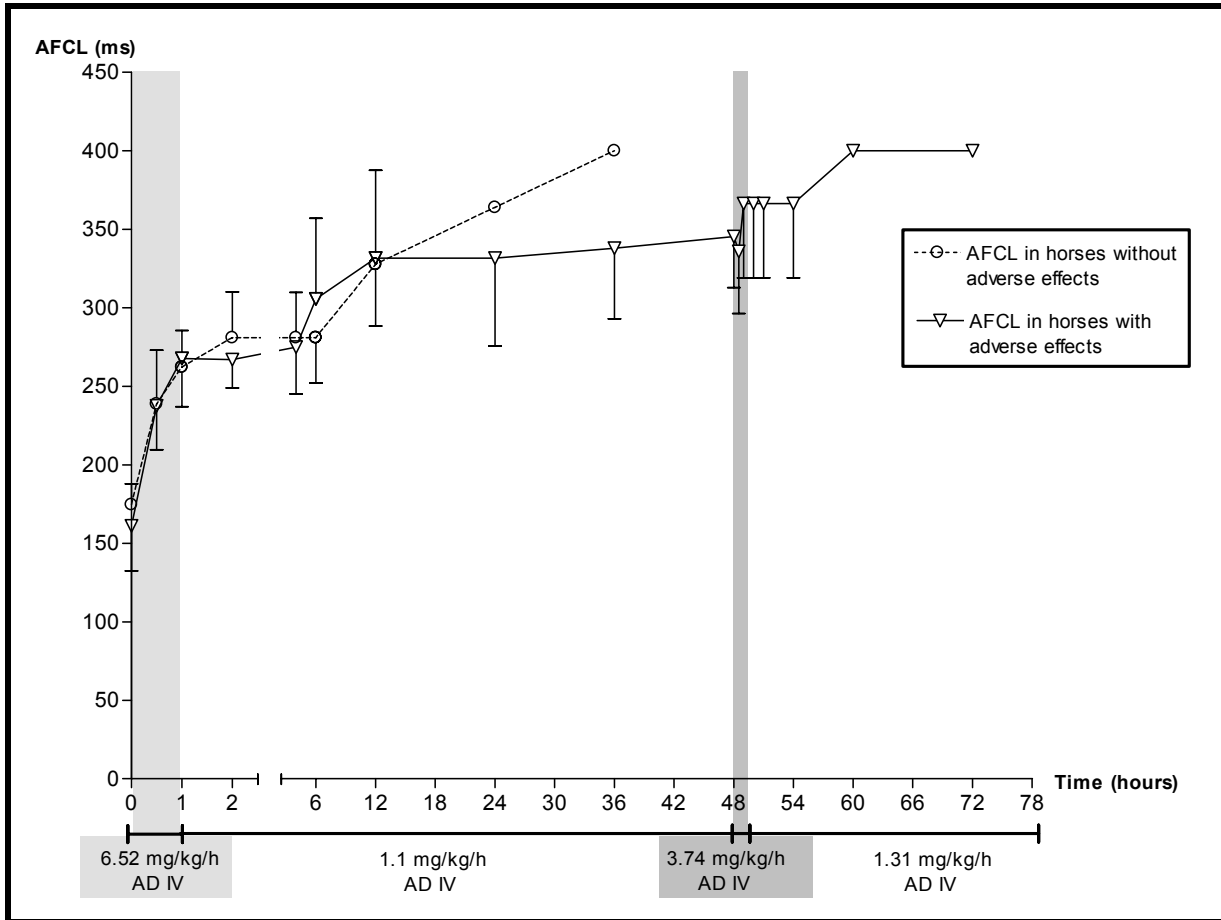
□ Horses that did not converted into sinus rhythm with the intravenous amiodarone treatment.

Figure 1. Mean (+/-SD) amiodarone (AD) and desethylamiodarone (DAD) plasma concentration ($\mu\text{g/mL}$) in patients with (n= 3 first 48 hours, n=2 from 48 to 54 hours and n=1 from 54 to 72 hours) and without (n=3 first 24 hours, n=1 at 36 hours) adverse effects, during treatment of a total of 6 horses.



During the first phase an amiodarone loading dose was administered at a rate of 6.52 mg/kg/h (\square) for 1 hour followed by an infusion at a rate of 1.1 mg/kg/h for the next 47 hours. In the second phase, a second loading dose at a rate of 3.74 mg/kg/h (\square) for 1 hour (from hour 48 to hour 49) was followed by an infusion at a rate of 1.31 mg/kg/h.

Figure 2. Mean (\pm SD) atrial fibrillation cycle length (AFCL) (ms) in horses with (n= 3 in first 48 hours, n=2 from 48 to 54 hours and n=1 from 54 to 72 hours) and without (n=3 first 24 hours, n=1 at 36 hours) adverse effects, during treatment of a total of 6 horses.



During the first phase an amiodarone loading dose was administered at a rate of 6.52 mg/kg/h (\square) for 1 hour followed by an infusion at a rate of 1.1 mg/kg/h for the next 47 hours. In the second phase, a second loading dose at a rate of 3.74 mg/kg/h (\square) for 1 hour (from hour 48 to hour 49) was followed by an infusion at a rate of 1.31 mg/kg/h.

Discussion

In human medicine, AD, is used to treat AF, atrial flutter and ventricular fibrillation with a moderate to good efficacy (Anderson, 1995; Kerin et al., 1996). This drug may terminate AF because it has similar actions to beta- and calcium channel blockers on the sino-atrial node, it increases the refractory period via sodium and potassium channel effects, and slows intracardiac conduction of the action potential via the sodium channel effects (Gill et al., 1992). Desethylamiodarone is an AD metabolite with a similar pharmacodynamic potency and toxicity as the parent drug (Gill et al., 1992).

Much contradictory information is available about AD and DAD plasma concentrations and the possibility of conversion. Some authors describe that the concentration of DAD is more important than that of AD (Balsler et al., 1991; Nattel et al., 1988). Others described a weak or even absent concentration-effect correlation (Rotmensch et al., 1984). In the present study, there was an apparent dose-response relationship. The horses with the highest AD/DAD plasma level showed adverse signs including diarrhea. However, in the previous equine study (De Clercq et al., 2006a), this effect-concentration correlation was not observed.

In human medicine, efficacy has been shown for AD plasma concentrations between 0.1–11.9 µg/mL (Latini et al., 1984); and a reference value of 2-2.5 µg/ml has been suggested as safe treatment with AD (Boppana et al., 1983; Haffajee et al., 1983; Mostow et al., 1982). Some authors describe that cardioversion occurs at an AD concentrations 0.6-3.0 µg/mL with a mean±SD of 2.5±1.3 µg/mL (Canada et al., 1983; Vardas et al., 2000). Based upon this information, the results from a pharmacokinetic study were used to calculate the infusion protocol for horses (De Clercq et al., 2006b). After 24 hours, the mean AD plasma level was 2.01±0.33 µg/mL (n = 6). At the end of the first phase, mean AD level was 2.64±0.58 µg/mL (n = 4). This rather high value was due largely to horse 5 which had a concentration of 3.43 µg/mL at that time. Two out of 6 horses reached the second phase and at the end of infusion, the concentrations were 2.51 and 2.57 µg/mL. The levels obtained approached the targets of 2.0 µg/mL AD after the first phase and 2.5 µg/mL after the second phase.

Nevertheless, some horses exceeded this therapeutic concentration, probably because of the pharmacokinetic variability of AD in horses. Some horses might have a lower volume of distribution and therefore show a higher plasma concentration. Also, because of the multi-compartmental pharmacokinetics of AD a prediction of plasma concentration is more difficult

and the importance of drug accumulation in long term infusion protocols can become important when the deep compartments are being filled (De Clercq et al., 2006b).

Some authors report no difference in AD plasma concentration between human responders and nonresponders (Haffajee et al., 1983). In the present study, an important difference of AD or DAD plasma levels during the first 24 hours of the infusion protocol was not observed between horses with or without development of adverse effects (Figure 1).

Many factors, including myocardial ultrastructure, AF duration, atrial size and electrophysiological properties might influence the success rate of pharmacological treatment. Long AF duration, large atrial size and short AFCL are all factors leading to increased AF stability (van Loon et al., 2000; Wijffels et al., 1995). All these factors could have been related to failure of treatment in these 3 horses.

In man, adverse effects due to chronic AD treatment include disturbances of the thyroid and pulmonary, hepatic, gastrointestinal (in particular dose related nausea), ocular, dermatological, epididymis and nerve effects (Gill et al., 1992). A few cases of hepatitis and one case report of anaphylaxis due to acute IV AD administration have been described (Agozzino et al., 2002; Bravo et al., 2005; Fransi and Briedis, 2004). Also, in dogs, adverse effects due to chronic AD treatment include hepatopathy, anorexia, lethargy, pruritis, neutropenia and thrombocytopenia (Calvert et al., 2000; Gilbert et al., 2000; Kraus et al., 2005; Oyama and Prosek, 2006). In a previous equine AD study, adverse clinical signs included an increase of total bilirubin and weakness of the hindlimb (De Clercq et al., 2006a). In the present study, 2 of 6 horses also showed a slightly increased serum total bilirubin concentrations and 3 of 6 horses had clinical signs of diarrhea. Malabsorption syndrome with severe diarrhea has been described in dogs after oral administration of sublethal doses of 100 mg of AD/kg for one month. Malabsorption syndrome was caused by partial villous atrophy and the accumulation of macrophages with dyslipidic inclusions (Vic et al., 1985).

It could be possible that a similar mechanism could have been present in our horses, but the exact mechanism of the appearance of diarrhea during short-term AD infusion is not described. The clinical signs in horse 6 were worse compared to the other 2 horses because of a concomitant Salmonella infection, of which it may have been a carrier. The bilirubin increase in horse 6 one week after termination of AD treatment, can be explained by the anorexic state of the animal. However, it is known that IV AD has complex pharmacokinetics (multi-compartmental) and that all mechanisms of toxicity and adverse effects are not yet

completely understood (De Clercq et al., 2006b; Kowey et al., 1997). Antioxidants reduce lysosomal phospholipidosis, which is a potential mechanism of AD toxicity (Honegger et al., 1995). Therefore, horses with adverse effects received vitamin E orally until relieve of the clinical signs.

A delayed therapeutic effect after withdrawal of therapy has been described in man and horses but was not observed in the current study (De Clercq et al., 2005; Latini et al., 1984).

AD treatment in horses with naturally-occurring chronic AF did not result in cardiac side effects, but noncardiac adverse effects included short-term hindlimb weakness (De Clercq et al., 2006a) and a 10-14 day period of diarrhea. These adverse effects only appeared in the horses that received an AD treatment for more than 36 hours.

In conclusion, the applied treatment protocol based upon pharmacokinetic data achieved clinically relevant concentrations of AD and DAD. A clinical benefit was observed in 6 horses based on intra-atrial electrogram and cardioversion was achieved in 3 horses. However, in the other 3 horses conversion was not achieved and adverse effects were identified. The therapeutic index of AD, based on the reported plasma levels, is likely to be low. Caution should be exercised in using this drug, particularly for prolonged infusions owing to the long half-life of the drug. In some horses conversion to sinus rhythm was possible, but further studies are necessary to evaluate a correct dosage regimen and the applicability of the drug in horses with chronic AF.

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SECTION 3

NON-PHARMACOLOGICAL TREATMENT OF ATRIAL FIBRILLATION IN HORSES

ELECTRICAL CARDIOVERSION

In human medicine, electrical cardioversion is highly effective to treat a wide range of arrhythmias, including atrial fibrillation (AF). When internal electrodes are used instead of external (skin) electrodes, energy requirements for successful cardioversion can be dramatically reduced. Because of the lower cardioversion threshold with internal cardioversion and because of failure of previous external cardioversion attempts in horses, the internal cardioversion technique to treat chronic AF in horses was further investigated in Chapter 7. As adapted cardioversion catheters for horses were not commercially available, custom-made catheters were used in this study.

CHAPTER 7

TRANSVENOUS ELECTRICAL CARDIOVERSION OF ATRIAL FIBRILLATION IN HORSES

TRANSVENOUS ELECTRICAL CARADIOVERSION OF ATRIAL FIBRILLATION IN SIX HORSES USING CUSTOM MADE CARADIOVERSION CATHETERS

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Summary

Pharmacological conversion of atrial fibrillation (AF) to sinus rhythm in horses can be difficult. The objective of this study was to investigate the feasibility of transvenous electrical cardioversion with custom made catheters in 8 horses, of which 3 had failed cardioversion using quinidine sulfate. Two cardioversion catheters and one pacing/sensing electrode were inserted via the right jugular vein and placed using ultrasound guidance into the left pulmonary artery, the right atrium and the right ventricle, respectively. Because immediate recurrence of AF was encountered in the second horse treated, pre-treatment with amiodarone was given to each of the remaining 6 horses. Induction of general anaesthesia was associated with dislocation of the cardioversion catheter in 3 horses, requiring a second catheterization procedure. During general anaesthesia, biphasic R wave synchronized shocks of up to 360 J were delivered between both cardioversion electrodes.

In 6 horses (75%), including 2 which had failed quinidine sulfate treatment, sinus rhythm was restored with a mean energy level of 295 ± 62 J. No side effects were observed. Blood analysis 3 hours after cardioversion revealed normal parameters, including cardiac troponin I values. Transvenous electrical cardioversion of AF with custom made cardioversion catheters can be considered as a treatment option for AF in horses, especially when conventional drugs fail.

Introduction

Atrial fibrillation (AF) is the most important clinically relevant arrhythmia in horses (Reef et al., 1988; Reef et al., 1995) and is known to occur because of atrial enlargement, myocarditis, autonomic nerve system imbalance, electrolyte or acid–base disturbances, administration of anesthetic drugs or tranquilizers, or for unknown causes (Reef and McGuirk, 2002). In contrast to humans and dogs, AF often occurs in horses without detectable cardiac pathology (Collatos, 1995; Deem and Fregin, 1982; Reef et al., 1988). Such horses have a rather good prognosis for cardioversion and they can generally return to their previous level of athletic performance after successful treatment.

After initiation of AF, rapid electrophysiological changes (within 1-2 days) (De Clercq et al., 2007a) and a decrease in atrial contractility (within 1-2 weeks) occur, leading to slight atrial dilatation (van Loon et al., 2001a, 2001b) and further AF stability (Allessie et al., 2002; Morillo et al., 1995; Schotten et al., 2003; van Loon, 2001b; Wijffels et al., 1995). In horses, it is generally believed that when AF persists for more than 3 months, especially when atrial dilatation is present, treatment becomes more difficult and AF recurrence is more common (Reef et al., 1988; van Loon et al., 2001a; 2001b).

The standard treatment for equine AF is quinidine sulfate (QS), but, in some countries the oral form is difficult to obtain and expensive. In addition, important QS-related side-effects may occur during treatment (McGurrin et al., 2003; Reef et al., 1988, 1995). Intravenous flecainide and amiodarone (AD) have been used, but these have shown only a moderate success rate and their use has often been associated with significant side effects (De Clercq et al., 2006, 2007b; Ohmura et al., 2000; van Loon et al., 2004). Therefore, alternative treatment options should be pursued in AF horses that do not tolerate medication or are drug-refractory.

In human beings, transthoracic cardioversion is highly successful in restoring sinus rhythm, but this procedure is difficult in horses because of the size of the thorax and the air-filled lungs between the cardioversion paddles. If external cardioversion fails in humans, internal cardioversion is an option. Here, one cardioversion catheter is generally placed in the high right atrium against the atrial wall and a second is positioned in the coronary sinus, which is in close contact with the left atrium. Low energy (<30 J) R wave synchronized shocks result in restoration of sinus rhythm in the majority of patients.

Since immediate recurrence of AF (IRAF) after successful electrical cardioversion is common, pre-treatment with anti-arrhythmic drugs is frequently used to prevent AF re-initiation (Duytschaever et al., 2000, 2002; Lévy et al., 1997; Ricard et al., 2003; Timmermans et al., 1998; Van Noord et al., 2002). Administration of AD has proven to be successful in preventing IRAF in human patients (Gorenek et al., 2006). Intravenous (IV) AD has also been described in horses for AF treatment and has been shown to produce rapid changes in electrophysiological properties (De Clercq et al., 2006, 2007b). Whereas long-term administration of AD (>36 hours) to convert AF to sinus rhythm in horses has been associated with side effects, short-term administration (<36 hours) revealed no such adverse effects (De Clercq et al., 2006, 2007b).

The application of electrical cardioversion to treat AF in horses has been described (Deem and Fregin, 1982; Frye et al., 2002; McGurrin et al., 2003, 2005a, 2005b; van Loon, 2001b; van Loon et al., 2005). Recently, McGurrin et al. (2005b) reported on the use of electrical cardioversion in a large number of horses with a success rate of 98%. Their catheter was 150 cm in length, 2.5 mm in diameter and had a coiled wire 9.5 cm long that served as a cardioversion electrode. The catheter was fluid filled, allowing intracardiac pressure registration. However, cardioversion catheters to perform transvenous electrical cardioversion in horses are not commercially available yet. The present report describes the results of transvenous electrical cardioversion of AF in 8 horses using custom made catheters.

Material and methods

Cases

AF was diagnosed in 4 French saddle horses, 2 Warmbloods and 2 Trotters that had been referred for exercise intolerance (horses 1–5 and 8), colic (horse 6) and arrhythmia (horse 7). The mean age, bodyweight and wither height were 9 ± 4 years, 567 ± 57 kg and 170 ± 6 cm, respectively. The horses were used for jumping, trotting or pleasure riding. The exact duration of AF was known in horse 4 (1.5 months), in horses 1 and 5 (2 months), in horse 3 (1 year) and in horse 2 (1.5 years). In the other horses, the duration of AF, based upon history and clinical examination, was supposed to be 3 months (horse 7) or 6 months (horses 6 and 8).

Horse 1 had been successfully treated previously with oral QS (22 mg/kg q 2 h) some 2 years previously, but developed severe tachycardia during treatment. This horse presented with recurrence of AF, with onset 2 months prior to examination. Three other horses (horses 4, 5 and 7) had recently been treated with QS but had failed to convert and showed obvious side effects including tachycardia, colic and diarrhea.

A clinical examination and a complete blood analysis were performed, including an assay for the cardiac biomarker troponin I using a luminescent immunoassay with a detection limit of 0.10 ng/mL (ADVIA centaur, Bayer Diagnostics). A full cardiac examination was undertaken including two-dimensional, M-mode and Colour Flow Doppler echocardiography (GE Vingmed CFM 800 SV) of the left atrial diameter at end-diastole and end-systole measured from the left cardiac window; aortic diameter in diastole; left ventricular internal diameter in diastole, and systole and left ventricular fractional shortening from the right cardiac window (Patteson et al., 1995). Colour Flow Doppler echocardiography was used to identify valvular regurgitation. Detailed patient information is shown in Table 1.

The owners were given 2 treatment options, including the administration of QS and the electrical cardioversion technique under general anaesthesia. Information was given about the success rate and the potential adverse effects of QS. The owners were also informed about the preliminary results on electrical cardioversion, the risk of general anaesthesia and the possible danger associated with transvenous electrical cardioversion, and the experimental design of the catheter. Written consent to perform electrical cardioversion was obtained from each owner.

Table 1. General information, auscultation of the heart, echocardiographic (2D, M-mode and Colour Flow Doppler) measurements of 8 horses.

Horse	1	2	3	4	5	6	7	8
GENERAL								
breed	SF	trotter	SF	trotter	SF	WB	WB	SF
age (years)	15	7	7	5	10	15	5	12
gender	gelding	mare	gelding	stallion	gelding	gelding	gelding	mare
bodyweight (kg)	600	523	592	457	627	557	557	615
height at the withers (cm)	180	167	168	162	176	168	174	170
referral reason	EI	EI	EI	EI	EI	colic	arrhythmia	EI
duration of atrial fibrillation (months)	2	18	12	1,5	2	6?	3?	6?
PREVIOUS TREATMENTS								
PT / SN / Re	Yes / SVT / SR	no	no	Yes / no / AF	Yes / colic / AF	no	Yes / D / AF	no
AUSCULTATION								
pathological murmur	1/6 HS MV	no	no	no	no	no	no	no
ECHOCARDIOGRAPHY (2D AND M-MODE)								
LADd (ref. 11.3-14.52 cm) ^o	14.48	11.33	14.45	13.01	13.78	13.44	12.74	12.22
LADs (ref. 11.22-14.48 cm) ^o	14.98	12.33	14.26	13.13	13.8	13.48	13.4	12.72
AOD (ref. 6.98-8.74 cm) ^o	7.00	6.99	7.00	7.34	7.48	7.49	7.23	7.60
LVIDd (ref. 9.90-12.20 cm) ^o	12.64	12.07	12.24	11.78	11.90	12.20	12.19	10.69
LVIDs (ref. 6.40-9.00 cm) ^o	7.70	7.93	8.12	8.99	7.24	8.80	8.11	6.44
FS (ref. 29.41-44.67%) ^o	39.09	34.29	32.43	31.26	39.13	34.00	40.80	39.78
COLOUR FLOW DOPPLER								
mitral valve	trivial	normal	normal	trivial	trivial	normal	trivial	trivial
aortic valve	trivial	normal	trivial	trivial	normal	trivial	normal	trivial
pulmonary valve	trivial	normal	normal	trivial	normal	normal	normal	normal
tricuspid valve	trivial	trivial	normal	trivial	trivial	normal	trivial	normal

SF: French saddle horse. WB: Warmblood horse. EI: exercise intolerance. HS: holosystolic murmur. MV: mitral valve area. PT: previous treatment with oral quinidine sulphate. SN: symptoms observed during quinidine treatment. Re: result of quinidine treatment. SVT: supraventricular tachycardia. SR: sinus rhythm. AF: atrial fibrillation. D: diarrhoea. LADd: left atrial diameter at end-diastole. LADs: left atrial diameter at end-systole. AOD: aortic diameter. LVIDd: left ventricular internal diameter in diastole. LVIDs: left ventricular internal diameter in systole. FS: left ventricular fractional shortening. ^o normal reference values according to Patteson et al. (1995).

Treatment regimen

Because the second treated horse (horse 2) showed IRAF within 6 min of electrical cardioversion, it was decided to pre-treat all subsequent horses with anti-arrhythmic drugs. Twenty-four hours before cardioversion, 6 horses (horses 3–8) received 6.52 mg/kg/h amiodarone (Cordarone 150 mg/3 mL, Sanofi-synthélabo) IV over 1 hour, followed by 1.1 mg/kg/h IV for 23 hours (De Clercq et al., 2007b). Electrocardiographs were recorded at baseline, 1, 2, 4, 6, 12 and 24 h. Blood was collected just before electrical shock delivery and the plasma analyzed for retrospective AD and its active metabolite desethylamiodarone by a validated high-performance liquid chromatography (HPLC) method combined with MS/MS detection (Maes et al., 2006). The limit of quantification for both components was 0.005 µg/mL.

In all horses, a 14 F introducer sheath (TriPort, Mansfield EP) was placed in the lower half of the right jugular vein. This introducer has three separate entrance ports, each with a haemostatic valve, for insertion of two custom made cardioversion catheters and one bipolar pacing catheter (Bipolar Intracardiac Electrode, USCI). The custom made catheter consisted of an insulated 0.35 mm steel wire and had a length of 180 cm. At the distal end, 12 cm of the insulation was removed to serve as cardioversion electrode. The distal end of the wire was isolated and smoothed with polyurethane in order to avoid tissue damage during catheter positioning (Figure 1).

Catheters were inserted and positioned in the standing sedated animal (detomidine, 10 µg/kg, Domosedan®, Pfizer Animal Health) under echocardiographic guidance: one cardioversion catheter in the left pulmonary artery (LPA) (Figure 2), one cardioversion catheter in the right atrium (RA), and a bipolar pacing catheter in the right ventricular apex. The tip of the pulmonary cardioversion catheter was advanced into the left pulmonary artery about 20 cm beyond the bifurcation of the pulmonary artery. During insertion of the pacing catheter, while the tip of the catheter was still located in the RA, the atrial fibrillation cycle length (AFCL) was measured (Programmer 9790, Medtronic) (van Loon et al., 1998). The mean AFCL was taken as the time between two successive atrial depolarization waves, measured in a window of 2–10 s.

After catheter placement in the standing horse, general anaesthesia was induced. Horses were pre-medicated with detomidine before induction, which was achieved with a combination of IV ketamine (Anesketin®, 2.2 mg/kg, Eurovet) and midazolam (Dormicum®, 0.06 mg/kg,

Roche). Induction was guided with webbing and horses were gently placed in left lateral recumbency. An orotracheal tube (30 mm diameter) was placed and isoflurane inhalant anaesthesia was given. Intermittent positive pressure ventilation was applied in all horses. Dobutamine was administered IV to maintain arterial blood pressure. A base apex ECG was connected to both a biphasic electrical defibrillator (Lifepak 20, Medtronic) and a pacemaker programmer (Programmer 9790, Medtronic). The custom made cardioversion catheters were connected to the defibrillator with the RA catheter as cathode and the LPA catheter as anode. The pacing catheter was connected to the pacemaker programmer to achieve ventricular pacing in case of temporary asystole (van Loon et al., 2005).

Catheter position was verified with ultrasound and modified if necessary. Shocks were delivered as biphasic truncated exponential waves and were synchronized with the R wave. Shock energy delivered to the horses was increased from 150 to 360 J in steps of 50 J until sinus rhythm was restored. An arbitrary 2-5 min delay was used between successive shocks to assess cardiac rhythm. Cardioversion catheters were removed 5-10 min after cardioversion to sinus rhythm.

After recovery, clinical status was followed and blood for complete blood analysis and cardiac troponin I assay was withdrawn 3 hours after the procedure. If sinus rhythm could not be restored during the first procedure, a second attempt at electrical cardioversion or oral QS treatment was made.

Figure 1. The distal end of the insulated (A) custom made cardioversion catheter has a cardioversion electrode (B) and a polyurethane-smoothened tip (C).

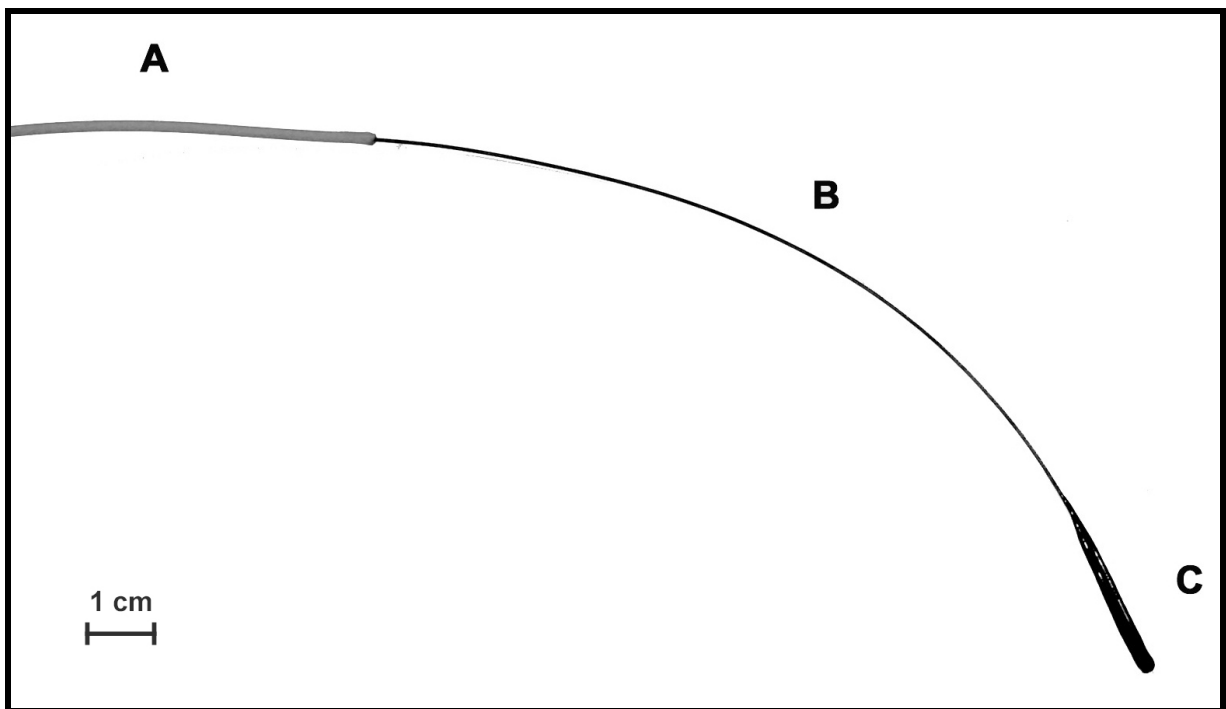


Figure 2. Right parasternal long-axis view with the right ventricular inflow-outflow of a horse. This echocardiogram was obtained with a 2.5 MHz sector-transducer at a displayed depth of 30 cm. The custom made catheter (arrows) can be observed in the main pulmonary artery (P) and is directed towards the left pulmonary artery (LPA). RA: right atrium, TV: tricuspid valve, RV: right ventricle, Ao: aorta, RPA: right pulmonary artery.

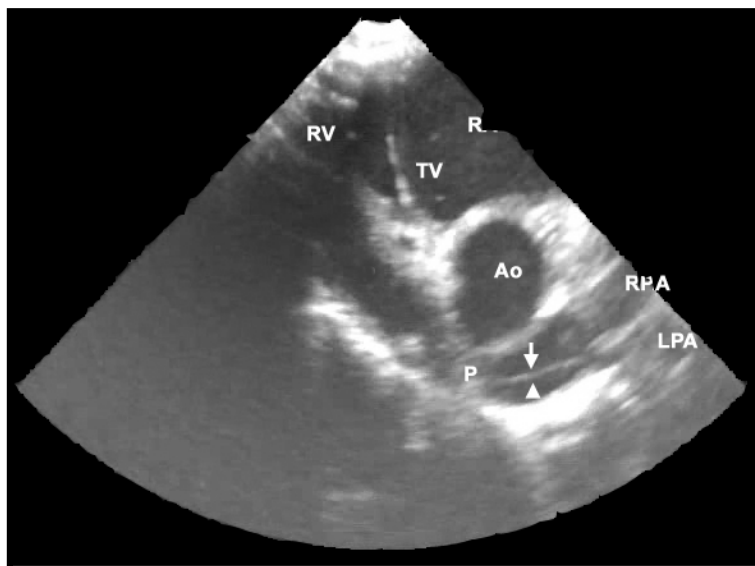
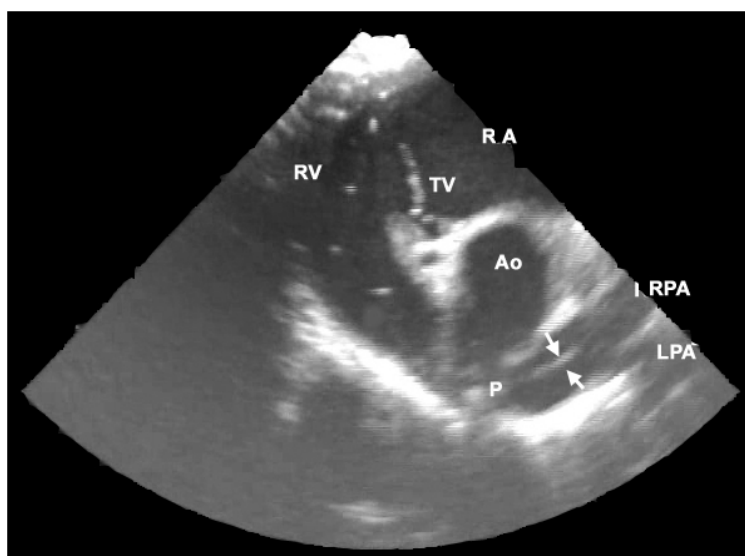


Figure 3. Right parasternal long-axis view with the right ventricular inflow-outflow of a horse. This echocardiogram was obtained with a 2.5 MHz sector-transducer at a displayed depth of 30 cm. An echogenic linear artefact resembling a catheter (arrows) can be observed in the main pulmonary artery (P). RA: right atrium, TV: tricuspid valve, RV: right ventricle, Ao: aorta, LPA: left pulmonary artery, RPA: right pulmonary artery.



Results

Before the procedure, biochemical and hematological parameters were all within normal reference ranges except for calcium and magnesium in two horses. Horse 3 presented a low magnesium level (2.0 mg/dL; ref. 2.2-2.8 mg/dL) and horse 4 had a low calcium level (10.2 mg/dL; ref. 11.2-13.6 mg/dL) (Carlson, 2002). Both horses received IV calcium and magnesium supplementation. Cardiac troponin I was normal in all horses (<0.15 ng/mL; Begg et al., 2006). The results of the cardiac examination were within normal limits except for horse 1 where the left atrial diameter at end-systole was 14.98 cm (ref. 11.22-14.48 cm) and left ventricular internal diameter during diastole was 12.64 cm (ref. 9.90–12.20 cm)(Patteson et al., 1995).

Repeated monitoring during and after AD treatment showed no adverse effects. Except for an occasional premature ventricular complex, no ECG abnormalities were seen during left pulmonary artery catheter placement. In some horses, visualization and positioning of the catheter in the left pulmonary artery was hampered due to echogenic linear artifacts in the main and right pulmonary artery. These artifacts were directed towards the right pulmonary artery (Figure 3). Mean (\pm SD) AD and desethylamidarone concentrations (μ g/mL) at the beginning of the shock delivery were 0.9 ± 0.2 and 0.1 ± 0.03 , respectively.

Details of baseline AFCL, cardioversion threshold, impedance, catheter position and total number of shocks delivered in each horse are shown in Table 2. In 5 of the 8 horses (horses 2-6) sinus rhythm could be restored (Figure 4) after a total of 3-18 shocks with a mean (\pm SD) energy level of 295 ± 62 J (range 200-360 J; Table 2). Lead impedance was between 48 and 72 Ω . However, after cardioversion and catheter withdrawal, horse 2 presented multiple atrial premature complexes. Six minutes after cardioversion an atrial premature complex resulted in recurrence of AF.

In 3 of the 8 horses (horses 1, 7 and 8), the cardioversion catheter from the LPA was displaced after induction of general anaesthesia. Several attempts to reposition the catheter during general anaesthesia failed, requiring termination of the cardioversion procedure. In 2 of the 3 horses (horses 7 and 8), a second electrical cardioversion attempt was performed after 10 days, which resulted in successful cardioversion in one horse (horse 7). No further cardioversion attempts were made in horse 2, the horse returned home as a broodmare.

Recovery from general anaesthesia was uneventful in all horses. One horse (horse 8) developed diarrhea and fever 4 days after the second cardioversion procedure. Three fecal samples were positive on Salmonella culture. The horse received IV fluid, antimicrobials and flunixin meglumine (Finadyne®, 1.1 mg/kg, Schering-Plough) and the diarrhea and fever resolved within 3 days. No further cardioversion attempts were made and the horse returned home as a broodmare. Haematological and biochemical parameters taken 3 hours after cardioversion, remained within normal ranges in all horses.

Ten days after the unsuccessful electrical cardioversion, horse 1 was treated with oral QS (22 mg/kg q 2 hours). After the second dose, treatment was terminated because of severe tachycardia (220 bpm). One hour later, cardioversion to sinus rhythm occurred. One week after electrical cardioversion, all converted horses were still in sinus rhythm and returned home.

Table 2. Catheter position, number of shocks, number of procedures and results are given for each horse.

Horse	1	2	3	4	5	6	7	8	Mean+/-SD°
AFCL (ms)	190	133	279 [†]	214 [†]	250 [†]	279 [†]	214 [†]	200 [†]	228+/-55
Cardioversion energy (Joule)	/	300	200	250	300	360	360	360*	295+/-70
Impedance (ohm)	/	72	60	48	65	60	58	54*	60+/-8
RA (cm)	/	56	63	56	72	79	65	50	65+/-9
LPA (cm)	/	116	143	117	165	157	135	105	138+/-20
Total number of shocks delivered	0	16	3	3	6	12	18	19	9+/-7
Number of cardioversion procedures	1	1	1	1	1	1	2	2	/
Result of 1 st procedure	/	SR / IRAF	SR	SR	SR	SR	AF	AF	/
Result of 2 nd procedure	/	/	/	/	/	/	SR	AF	/

■ Horses converted to sinus rhythm with the electrical cardioversion protocol.

[†] AFCL measured after a 24-hour amiodarone infusion protocol

* Highest energy level and impedance are given, although sinus rhythm could not be restored.

° Values of horse 1 and 8 not included

SR: sinus rhythm.

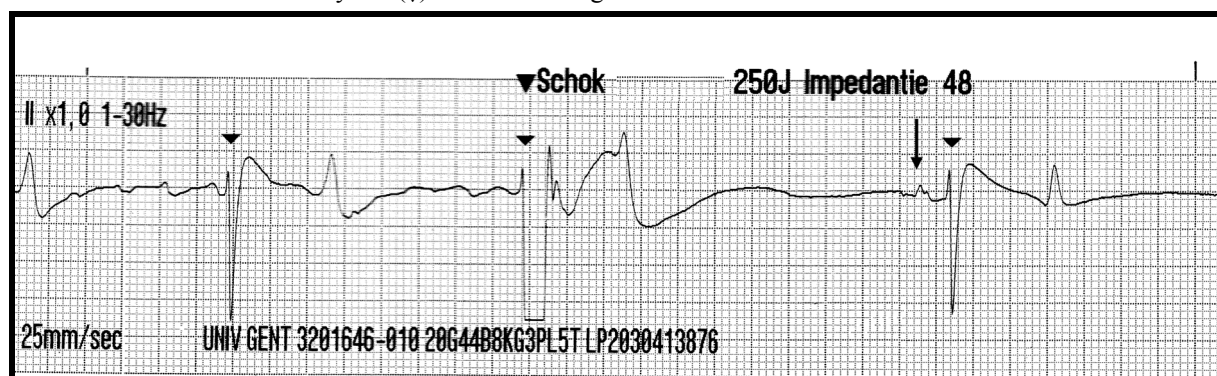
RA: catheter depth in the right atrium from the lower half of the jugular vein

LPA: catheter depth in the left pulmonary artery from the lower half of the jugular vein.

IRAF: immediate recurrence of atrial fibrillation after successful electrical cardioversion.

AF: atrial fibrillation.

Figure 4. A base apex electrocardiogram during delivery of a 250 joule R wave synchronized shock (▼) in horse 4 shows restoration of sinus rhythm (↓). Lead recording is 25 mm/s and 1mV/cm



Discussion

Treatment of AF in horses without detectable cardiac pathology is recommended as there is a good prognosis (Collatos, 1995; Deem and Fregin, 1982; Reef et al., 1988). In our patient data, only one horse had a left atrial diameter at the end of systole and a left internal diameter during diastole that were slightly out of the normal reference range described by Patteson et al. (1995). However, this was thought to be partially related to the size of the animal.

Disturbances in electrolyte concentrations are supposed to be a contributing factor to the occurrence of AF (Reef and McGuirk, 2002). Electrolyte values were determined in each horse before the procedure and normalized if necessary, although the exact effect on the outcome was unknown.

In human patients, electrical cardioversion is highly effective in converting AF to sinus rhythm. The associated risk for embolic complications makes echocardiographic monitoring and anti-coagulation treatment mandatory prior to the procedure (Capucci et al., 2000; Ricard et al., 2003). Pre-treatment with anti-arrhythmic drugs is recommended because cardioversion recurrence of AF is common from between <1 min up to 2 weeks (Chugh et al., 2004; Duytschaever et al., 2000, 2002; Gorenek et al., 2002; Joglar and Kowal, 2004; Lévy et al., 1997; Ricard et al., 2003; Sarubbi et al., 1998; Timmermans et al., 1998; Van Noord et al., 2002). In horses, transvenous electrical cardioversion has been applied to cardiovert AF (Deem and Fregin, 1982; Frye et al., 2002; McGurrin et al., 2003, 2005a, 2005b; van Loon, 2001b, 2005) but as thromboembolic events have not been associated with AF in horses, anti-coagulation therapy is not indicated.

To the authors' knowledge, IRAF has not yet been reported in horses. This might be explained by the fact that during conventional QS treatment, the therapeutic drug level at the time of conversion prevents AF from re-occurring immediately. During electrical cardioversion without anti-arrhythmic drugs, IRAF is more likely to occur because of the high vulnerability of the atria at the time of conversion (Duytschaever et al., 2000, 2002). Because horse 2 relapsed to AF within 6 min of conversion, it was decided to load all subsequent horses with AD in the 24 hours preceding the electrical cardioversion procedure to reduce the risk for IRAF.

In human patients, AD has proved efficacious in preventing IRAF (Gorenek et al., 2006) and therapeutic AD plasma concentrations for cardioversion in humans range between 0.1 and

11.9 µg/mL (Latini et al., 1984). Some authors have reported that cardioversion occurs at an AD concentration between 0.6 and 3.0 µg/mL, with a mean±SD of 2.5±1.3 (Canada et al., 1983; Vardas et al., 2000), although Rotmensch et al. (1984) describe a weak or even absence of any concentration-effect relationship. Our goal in administering AD to horses was not to convert AF to sinus rhythm, but to change the electrophysiological properties of the atria as AD prolongs AFCL quickly (1–2 hours) in horses with chronic AF (De Clercq et al., 2006, 2007b).

In contrast with a prolonged IV administration, the 24-hour loading with amiodarone did not result in side-effects (De Clercq et al., 2006, 2007b). The diarrhea occurring in horse 8 after cardioversion was not thought to be directly caused by AD because of the late occurrence and the positive fecal Salmonella culture.

McGurrin et al. (2005b) have described a success rate of 98% for electrical cardioversion in horses, but equine cardioversion catheters are not yet commercially available. Human cardioversion catheters are only 120 cm long, which is usually insufficient to place the catheter tip into the equine LPA. Therefore, we developed custom made catheters with a length of 180 cm. Electrode placement into the LPA is recommended because, when combined with a right atrial electrode, the current between both electrodes will “cover” more atrial myocardium, so lowering the threshold for cardioversion (Alt et al., 1997a, 1997b; Saksena et al., 1995).

LPA catheter placement was hampered by a rather poor visibility, due to the depth of the thorax and the small diameter of the catheter. In addition, after passing through the RA, RV and pulmonary artery, the catheter entered more easily the right branch of the PA instead of the left. Repeated insertion and withdrawal while twisting the catheter was necessary in order for it to enter the LPA.

In most horses, the distal part of the catheter, located in the LPA, could not be distinguished on ultrasound, but the more proximal part in the main pulmonary artery was clearly directed towards the LPA (Figure 2). In some horses, visualization and positioning of the catheter in the LPA was hampered due to echogenic linear artifacts in the main and right pulmonary artery. Fluoroscopic guidance of catheter placement, as used in humans, is difficult in horses because of their size. Exact placement of the catheter could not be guided by radiography due to technical limitations. Blood pressure monitoring was not possible with our custom made catheter, which challenged catheter placement rather more than was described by McGurrin et

al. (2005b). A pacing catheter was placed in the right ventricle as back-up because transvenous electrical cardioversion can provoke temporary complete atrioventricular block (van Loon et al., 2005). Webbing during the induction of general anaesthesia did not seem to prevent displacement of the LPA catheter, as we found this occurred in three horses.

Shocks were delivered synchronously with the R waves to avoid ventricular arrhythmias (McGurrin et al., 2003). Mean cardioversion energy to restore sinus rhythm was higher in this study compared to the results (295 ± 62 J vs. 162 ± 10 J) described by McGurrin et al. (2005b). This difference might be explained by a different electrode position in the LPA, the smaller diameter and therefore the smaller surface of the cardioversion electrode, or indeed by other individual factors. Nevertheless, despite these higher cardioversion thresholds and number of shocks delivered, cardiac troponin I concentrations remained within normal limits.

In the present study, the use of custom made catheters allowed us to undertake successful transvenous electrical cardioversion in six horses. The small diameter and the lack of intracardiac pressure monitoring challenged correct catheter positioning. High energy levels and a large number of shocks were required to achieve cardioversion, but were not associated with an increase in cardiac troponin I concentrations. Further adaptations of the catheter design to increase the surface of the cardioversion electrode and to facilitate catheter visibility and positioning are necessary to increase the success rate.

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GENERAL DISCUSSION

This thesis studies the pathophysiology of short (7 days) and long-term (4 months) atrial fibrillation (AF) in horses. It also describes alternative treatment possibilities for chronic AF in horses.

A. Pathophysiologic aspects of AF in horses

Since it is difficult to obtain information about the pathophysiology of AF in horses suffering from naturally-occurring AF, an experimental model was used in this PhD thesis. To realize this experimental model, a pacemaker and a neurostimulator were implanted in normal healthy horses as described by van Loon (2002). Although the technique for pacemaker implantation was similar as described in ponies, some horses presented an important increase in threshold value especially during the AF study period. Presumably pacemaker electrodes were more likely to wobble in the large equine atrium, which may result in irritation at the anchored tip, followed by formation of fibrosis. Especially during induction of AF, rapid movement of the atrial myocardium contributed to the swinging effect on the electrode. However, no post mortem data are available to prove this hypothesis. Because of an important raise in threshold above maximal pacemaker output, one horse was excluded from the AF study and the pacemakers were removed.

Similar to ponies, dogs and goats, a rapid atrial electrical and contractile remodeling was observed in both study protocols of our horses (Morillo et al, 1995; van Loon et al., 2001; 2002; Wijffels et al., 1995). After restoration of sinus rhythm reverse remodeling occurred within 2 days and 2 months for the 7-day and the 4-month AF protocol, respectively.

An increase in AF vulnerability has been observed in ponies, goats and dogs (Morillo et al, 1995; van Loon et al., 2001; 2002; Wijffels et al., 1995). However, long-term burst-pacing protocols resulted in short-term AF periods in dogs and ponies but in long-term AF periods in goats (Allessie et al., 2002; Morillo et al., 1995, van Loon et al., 2001; 2002). In our study, each horse developed self-sustaining persistent AF during the chronic protocol and even 2 of the 6 horses during the short-term AF protocol. Many factors play a role in the induction and maintenance of AF but the theory of Moe (1962) might at least in part explain why smaller animals with the presence of a small atrial area have less tendency to fibrillate than larger animals with a much larger atrial surface area. This investigator described that the presence of AF depends on the critical numbers of re-entry circuits that an atrium can contain. Allessie

and coworkers (1985) described that the critical numbers of wavelets for perpetuation of AF were between 3 and 6. However, not only atrial size but also the size of a re-entry circuit is important in the induction and perpetuation of AF. This size is determined by the wavelength (WL) of the re-entrant circuit and is defined by the atrial effective refractory period (AERP) and the conduction velocity (CV) ($WL = AERP \cdot CV$). Due to technical limitations in horses, no information is available about CV. Subsequently, the appearance of a “relatively” large atrium and/or a decrease of the wavelength, i.e. a short AERP or a decrease in CV, are factors that contribute to the induction and persistence of AF. All the different parameters need to be interpreted as one entity to understand the pathophysiology of AF.

Human researchers have suggested that electrical remodeling was an important factor of increase in AF vulnerability that sometimes may lead to persistent AF (Wijffels et al., 1995). However, since shortening of the AERP and a new steady state level appear very rapidly, other factors must be involved to explain the decreased efficacy of cardioversion and the reoccurrence of AF after conversion in patients with chronic AF. Besides electrical remodeling, important contractile remodeling occurs during AF, which could also influence AF susceptibility during AF. During AF an overload of intracellular calcium occurs which results in a downregulation of the L-type calcium channels which finally leads to a decrease in sarcolemmal calcium influx (Allessie et al., 2002; Courtemanche et al., 1998). The contractile dysfunction may lead to atrial paralysis and an increase in the atrial compliance. Subsequently, this may induce atrial dilatation with an increase of AF duration (Schotten and Allessie, 2001). A slight increase in atrial diameter was observed in our long-term AF study in horses.

Only during the first days of AF a slightly increased heart rate was observed which might explain that atria have less time to fill and therefore contain less volume of blood. In human patients and dogs, AF is characterized by an increased ventricular rate (Hnatkova et al., 1998; King et al., 2002; Miller et al., 1999; Silbauer and Sulke, 2007). However, most likely, only a slight increase in heart rate was observed during the first 7 days of AF because horses have a high vagal tone. From 7 days onwards a normal heart frequency was observed in the horses. The latter observation might be explained by a decrease in atrial fibrillation cycle length (AFCL). As in human medicine, a long AFCL represents more atrial flutter and coincides with a higher ventricular rate than in atrial fibrillation because of less concealed conduction at the level of the atrioventricular node (Zipes, 1992). The influence of the parasympathicus in

horses with AF will play a role in a less elevated ventricular response. Therefore, in contrast to human patients, rate control is not necessary in horses suffering from AF (Herzog et al., 2005; King et al., 2002).

During the last years, besides the wavelet theory described by Moe (1962), new information about triggering factors has become available. Indeed, in human medicine, the presence of rotors and spiral waves has been described. A rotor is a stable rotating reaction around a pivot point which gives rise to spiraling wave fronts. Under adequate circumstances, these rotors may give intermittent or permanent high frequency activation which conducts to the other atrium and therefore induces and maintains atrial fibrillation. The presence of one or more rotors has been described in both atria but most dominant rotors are localized at or near the pulmonary veins and the posterior left atrial wall (Jalife, 2003). Currently, no information is available about these findings in horses.

B. Treatment of AF in horses

In many horses treatment of AF is recommended because horses suffer, in general, from lone AF. After conversion to sinus rhythm they usually return to their previous level of performance (Reef et al., 1988). Until now, *quinidine sulphate* (QS), a class I anti-arrhythmic drug, has been the drug of choice for pharmacological treatment of AF in horses. It has an efficacy of 85% in horses with AF duration < 2 months and without the presence of detectable cardiac disease. However, in up to 76% of treated horses this drug is associated with serious cardiac and non-cardiac side effects including urticaria, nasal edema, colic, diarrhea, laminitis, polymorphic ventricular tachycardia, anaphylactic shock, syncope or sudden death (Reef et al., 1988; 1995). Recently, QS has become more difficult to obtain in some countries (McGurrin et al., 2003). Therefore, we decided to explore alternative treatment options of AF in horses (section 2 and 3). We chose to study horses with chronic AF because drugs which might be useful in horses with acute AF are not necessarily effective in cases with chronic AF.

Flecainide, a class I anti-arrhythmic drug, has been shown to be efficacious in horses with acute experimentally-induced AF but not in horses with chronic AF because of the appearance of potentially dangerous ventricular dysrhythmias (Ohmura et al., 2000; van Loon et al., 2004). During the research work for this thesis, we have observed the dangerous adverse

effects of intravenous flecainide in an acute experimentally induced AF. In one specific horse we observed the appearance of very wide QRS complexes ending in a fatal ventricular fibrillation and asystole. In human medicine, administration of flecainide has been described as being safe in patients who have no structural cardiopathy, and is frequently used in the prevention of atrial fibrillation. However, in a patient with a history of infarction and conduction disorders, its proarrhythmia may be lethal (The Cardiac Arrhythmia Suppression Trial Investigators, 1989). Flecainide-induced torsades des pointes may appear if combined with the use of other anti-arrhythmic drugs and/or the presence of electrolyte disorders (Nogales et al., 2007; Ohki et al., 2001; Thevenin et al., 2003). No electrolyte disturbances were present and macroscopic and microscopic examination could not reveal any underlying myocardial disease in our horse.

In human medicine, *propafenone* is used for treatment of supraventricular and ventricular arrhythmias and several studies describe that this drug is more or as efficient as QS and other class I or class III anti-arrhythmic drugs for prevention and suppression of arrhythmias (Dinh et al., 1985; Faucher et al., 1986; Klempt et al., 1982; Odeh et al., 2000; Waleffe et al., 1981). In addition, the use of intravenous propafenone has already been described for treatment of ventricular arrhythmias in horses (Reef, 1999). Therefore, we have investigated the treatment possibility of propafenone in chronic naturally-occurring or experimentally-induced AF in horses.

Although the therapeutic propafenone plasma concentration is very variable and ranges from 20-200 ng/mL in humans and 64-1044 ng/mL in dogs, in all treated horses the human reference ranges could be achieved and a prolongation in AFCL could be measured (Bifi et al., 1999; Chan et al., 1989; Connolly et al., 1983; Karagueurzian et al., 1982; Steurer et al., 1991). However, restoration of sinus rhythm was not observed in the treated horses. Side effects as described in human medicine due to acute or chronic doses of propafenone including cardiac, hepatic, cholestatic, dermatologic or nerve disorders, were not observed in our study (Cocozzella et al., 2003; Gandolfi et al., 2001; Huang et al., 2005; Odeh et al., 2000; Spingler et al., 1992; Wiesfeld et al., 2006). During administration of propafenone infusion, horses had an increased reaction to auditory and visual stimuli.

Because of the very low success rate of flecainide and propafenone, both class I anti-arrhythmic drugs, we chose to change the direction of our research into the use of a class III anti-arrhythmic drug. *Amiodarone* (AD) is a class III anti-arrhythmic drug and is described as

being efficacious and safe for treatment of atrial fibrillation, atrial flutter and ventricular fibrillation in human patients (Anderson et al., 1995; Kerin et al., 1996; Kodama et al., 1997; Podrid, 1995; Singh, 1995). This drug inhibits outward potassium currents resulting in prolongation of the repolarization time which may lead to AF termination. The dose used in a first treatment protocol was extrapolated from reported human doses to the weight of a horse (Kerin et al., 1996; Kochiadakis et al., 1999a, 1999b; Vardas et al., 2000). In human patients, the therapeutic AD plasma concentration is very variable and ranges between 0.1-11.9 µg/mL but a reference value of 2-2.5 µg/mL has been suggested as being safe (Boppana et al., 1983; Haffajee et al., 1983; Latini et al., 1984; Mostow et al., 1982). With this treatment protocol a prolongation in AFCL was observed leading to termination of AF in 4 of the 6 horses. Adverse effects such as thyroid, pulmonary, gastrointestinal, ocular, epididymal and dermatological disturbances that occur in human patients were not encountered in the treated horses. Only nervous disorders due to slowing down of the sensory and motor nerve conduction velocity have been observed in some of the horses using the described treatment protocol (Besser et al., 1994; Gill et al., 1992). A variability in blood-nerve barrier efficacy between individuals has been suggested in man and might also be an explanation why this problem has been seen only in some of the treated horses (Santoro et al., 1992). In addition, a slight increase in total bilirubin was present in some horses but normalization occurred after termination of AD treatment. In humans and dogs, a few cases of hepatic disorders after acute IV and after chronic AD treatment have been described (Agozzino et al., 2002; Bravo et al., 2005; Calvert et al., 2000; Fransi and Briedis, 2002; Gilbert et al., 2000; Kraus et al., 2005; Oyama and Prosek, 2006). A major advantage compared to QS and flecainide was that none of the horses showed any cardiac adverse effects.

This information suggested that AD could be used as an alternative treatment option for naturally-occurring AF in horses. Since the encouraging results and in order to increase success rate we investigated the pharmacokinetics and the bioavailability of intravenously and orally administered AD in horses.

As described in human studies (Chow, 1996), no changes in respiratory rate, P-R interval, QRS duration or Q-T interval and no arrhythmias were observed in the studied group of horses. These findings were in contrast to the prolonged QT-interval observed after a single dose of AD in ponies (Trachsel et al., 2004). In discrepancy to the findings in dogs but in accordance with the results in humans and ponies, a moderate increase in heart rate after a

single IV AD bolus was observed in horses (Connolly et al., 1984; Trachsel et al., 2004; Wellens et al., 1984). This increase in heart rate might be related to the occurrence of hypotension after IV administration most likely due to the solvent as suggested in human data (Gill et al., 1992; Vardas et al., 2000; Wellens et al., 1984). As described for dogs and humans, the pharmacokinetics of IV AD fitted best with a multi-compartmental model (Plomp et al., 1984; Saavedra et al., 1999). Values of mean volume of distribution (31.1 L/kg) and elimination half-life (51.1 hours) of AD were in accordance with those described for humans (Latini et al., 1983, 1984; Plomp et al., 1984; Riva et al., 1982). Trachsel et al. (2004) reported a much longer elimination half-life (16.3 days) in ponies but the difference is probably related to the fact that they used a mathematical based pharmacokinetic model.

As observed in humans, dogs and rats, oral AD administration poorly fitted the compartmental model. The bioavailability in horses was less than 50% (19.4%) comparable to that reported in dogs and rats, whereas in human patients, values range from 22-86% (Andreasen et al., 1981; Broekhuizen et al., 1969; Canada et al., 1983; Haffajee et al., 1983; Holt et al., 1983; Plomp et al., 1984; Riva et al., 1982). These findings suggest that large amounts of oral drugs would be necessary to obtain therapeutic plasma concentrations in horses. This low bioavailability might explain why one horse treated for AF with oral AD for a period of 30 days could not be converted to sinus rhythm (Guglielmini et al., 2003).

In contrast to the findings of Trachsel and coworkers (2004), no renal excretion of the drug appeared in the horses. Again, this difference might be explained because Trachsel et al. (2004) used a physiologically based pharmacokinetic model to estimate pharmacokinetic parameters and to predict elimination of AD in urine.

Based upon the pharmacokinetic findings, an adapted intravenous AD protocol was calculated to achieve the advised therapeutic levels of 2 to 2.5 µg/ml (Boppana et al., 1983; Haffajee et al., 1983; Mostow et al., 1982). Although target AD levels were achieved by slightly increasing the AD dose, some horses exceeded this therapeutic AD plasma concentration. With this protocol no cardiac side effects or nerve disturbances were observed but diarrhea occurred in three of the six horses in which sinus rhythm could not be restored. The appearance of malabsorption syndrome with severe diarrhea has been described in dogs after chronic oral administration of sublethal doses of AD due to partial villous atrophy and the accumulation of macrophages with dislipidic inclusions (Vic et al., 1985). A similar mechanism might have been responsible for the diarrhea in our horses, although they were

only treated for a short period of time with much smaller doses. However, no postmortem data are available to prove this theory.

Prediction of AD plasma concentration and its impact on successful treatment remains very difficult because of its multicompartmental pharmacokinetics especially in long-term infusion protocols. It was concluded that AD could be used as an alternative treatment but that adverse effects were likely, particularly with prolonged infusion time (>36 hours).

Because of the moderate success rate of the previously studied pharmacological treatments and the known efficacy of *internal electrical cardioversion* of AF in human patients, the applicability of the latter technique in horses was studied. Because adapted cardioversion catheters for horses are not commercially available, we examined the use of a custom made cardioversion catheter for internal cardioversion in horses. However, we encountered the complication of immediate recurrence of AF (IRAF) in one horse. In human medicine, IRAF after successful electrical cardioversion is common and pre-treatment with anti-arrhythmic drugs is frequently used for prevention of AF re-initiation within <1 min to 2 weeks (Chugh et al., 2004; Duytschaever et al., 2000, 2002; Gorenek et al., 2002; ; Joglar and Kowal, 2004; Lévy et al., 1997; Ricard et al., 2003; Sarubbi et al., 1998; Timmermans et al., 1998; Van Noord et al., 2002). Administration of AD has proven to be successful in preventing IRAF in human patients (Gorenek et al., 2006). As our previous studies indicated that short-term (< 36 hours) AD infusion was not associated with cardiac or non-cardiac side effects all subsequent horses were loaded with a 24-hour AD infusion. The pharmacokinetic-based AD dose was chosen to achieve higher plasma concentrations at the moment of electrical cardioversion. The goal of the AD administration in these horses was thus not to convert AF to sinus rhythm, but to change electrophysiological properties of the atria for preventing IRAF. IRAF has not yet been described in horses which might be explained by the fact that during conventional QS treatment, the therapeutic plasma concentration at the time of conversion and the gradual decrease afterwards protect the atria from IRAF. Until now, in the horses, pre-loaded with an AD infusion, we have not encountered the problem of IRAF anymore.

In conclusion, this thesis provides background information about the pathophysiology of recent-onset (paroxysmal) and chronic AF. In addition, it gives information about alternative treatment possibilities for chronic AF in horses. The currently used intravenous propafenone

treatment achieved therapeutic propafenone plasma concentrations without severe cardiac or non-cardiac side effects but sinus rhythm could not be restored in horses with chronic naturally-occurring or experimentally-induced AF. The use of IV flecainide in horses with chronic AF as well as in recent-onset AF is strongly discouraged because of its pro-arrhythmic effects.

Intravenous AD can be used as an alternative treatment option with moderate success rate but non-cardiac side-effects such as hind limb weakness and diarrhea might be expected.

Internal electrical cardioversion is possible with custom made electrical cardioversion catheters with a good success rate and might be advised especially when conventional drugs fails. A combined use of anti-arrhythmic drugs and electrical cardioversion might be recommended to prevent the occurrence of IRAF.

This work gives important new information about the pathophysiology and treatment possibilities of AF in horses.

Future prospects

From human patients and from experimental work in dogs and goats, it is known that, besides electrical and contractile remodeling, also structural remodeling plays a major role in the pathophysiology of AF. It has been described that during atrial fibrillation adaptive reversible responses occur at the ionic level within 30 minutes or at the cellular level within one week (Ausma et al., 2001; Goette et al., 1996). However, if AF exists for a longer period of time (> 5 weeks) structural changes might occur such as a change in the distribution of the protein connexin 40, myolysis characterized by disruption of the sarcoplasmic reticulum and accumulation of glycogen (Ausma et al., 1997; Van der Velden et al., 1998). Some studies also showed alterations in gap-junctions, which influences conduction velocity (Ausma et al., 1997; Elvan et al., 1997; Van der velden et al., 1998), or evidence of apoptosis and fibrosis (Li et al., 1999). No information is currently available about whether or not structural changes also play a role in horses. Compared to human patients, horses generally present AF for a much longer time (several months versus a few days or weeks) and as such, structural changes might become even more important. On the other hand atrial structural lesions (e.g. fibrosis) might also be a predisposing cause of AF in horses and might influence response to treatment

and risk for AF recurrence after conversion. As these structural lesions are unlikely to be detected by cardiac ultrasound these horses would erroneously be classified as ‘lone’ fibrillators.

Although the exact mechanism of AF is still not fully understood, the left atrium and pulmonary veins are known to be an important ‘source’ for AF in human patients. Especially sleeves of myocardium extending into the pulmonary veins are thought to be important triggers for AF. The importance of these structures in the initiation or maintenance of AF in horses is currently unknown. Comparison of post mortem examination of normal horses and horses with chronic AF should be able to clarify whether structural lesions are involved in horses with AF and whether pulmonary sleeves of atrial myocardium are also found in horses.

Concerning the treatment of AF, transvenous electrical cardioversion of AF is becoming increasingly important as it shows to be well tolerated and has a good efficacy. Further studies are still necessary to reveal the optimal electrode position within the heart and to explore the advantages of concurrent anti-arrhythmic drug treatment to improve success rate, decrease cardioversion threshold and to minimize early recurrence of AF after successful cardioversion.

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Atrial fibrillation (AF) is the most common, clinically important cardiac arrhythmia in horses. Nevertheless, information regarding the pathophysiology of this complex arrhythmia remains limited. In human medicine, dogs, goats and ponies, electrical changes during AF have been investigated. However, limited information about electrical changes during short-term AF in horses is available. These data might give new information about the pathophysiology of paroxysmal AF in horses.

The standard drug, quinidine sulphate, used in horses for treatment of AF, becomes more and more difficult to obtain in some countries. Despite a variety of treatment options in human medicine, information regarding alternative pharmacological or non-pharmacological treatment protocols in horses is scarce.

In this thesis a general introduction discusses in a first section the pathophysiology of AF and in a second part the underlying mechanisms of the different classes of anti-arrhythmic drugs that could potentially be used to treat AF.

The research of the thesis consists of three major sections. The **first section** (Chapters 1-2) describes the electrophysiological, contractile and dimensional changes associated with short-term and long-term AF in horses. In the **second section** (Chapters 3-6) new pharmacological approaches for the treatment of AF in horses are being explored. The **third section** (Chapter 7) describes the results of a new non-pharmacological treatment of AF in horses.

In the **first chapter**, 6 horses were instrumented with a pulse generator and a pacemaker. The pulse generator was connected with an atrial lead and the pacemaker with an atrial and ventricular lead. With the pulse generator it was possible to induce and maintain AF with burst stimulations for a short period (7 days). With the pacemaker, electrophysiological measurements including atrial and ventricular effective refractory period, could be measured before, during and after this short-term AF period. At the same time, echocardiographic

measurements including atrial diameters and contractility were assessed. In 2 horses, AF became persistent necessitating pharmacological cardioversion.

In all horses, atrial electrical and contractile remodeling was observed very quickly (24 hours) during maintained AF. But after restoration of sinus rhythm, values returned quickly (48 hours) to baseline. No obvious changes could be observed at the level of the ventricle and atrial diameters and surface areas.

Clinical relevance:

These findings support the concept that early conversion of AF is preferable and that an early return to training is possible after treatment of a short AF episode.

In the **second chapter**, in a similar setup, AF was maintained in 4 healthy horses and electrophysiological and echocardiographic measurements were followed.

In all horses, atrial electrical and contractile remodeling was observed very quickly (24 hours) during maintained AF, however after restoration to sinus rhythm, values returned more slowly back to baseline (2 months). No obvious changes could be observed at the level of the ventricle.

Clinical relevance:

These findings suggest that after 4 months of AF, it takes 2 months for the atrium to regain normal size and function.

In **Chapter 3**, the applicability of propafenone (Rhythmnorm®), a class I anti-arrhythmic drug, was investigated for its use in horses with chronic AF. Two horses with naturally-occurring AF and 4 horses with experimentally induced AF received a treatment protocol as suggested in human medicine. Horses received 2 mg/kg of propafenone IV over 15 minutes. If AF persisted 20 minutes after this bolus, a continuous infusion of 7 µg/kg/min of propafenone was given for another 85 minutes. Infusion was discontinued when conversion was achieved or when any side effects were observed.

The current protocol did not result in cardiac or non cardiac side effects. An increase of atrial fibrillation cycle length was observed but conversion to sinus rhythm did not occur.

Clinical relevance:

Propafenone, given at this dose, is not likely to convert chronic AF in horses.

The **fourth chapter** describes the applicability and adverse effects of intravenous amiodarone (AD) (Cordarone®), a class III anti-arrhythmic drug, in horses with chronic AF. Six horses received a treatment protocol as suggested in human medicine adapted to the weight of a horse. Horses received 5 mg/kg/h AD IV during 1 hour followed by 0.83 mg/kg/h IV for 23 hours and then 1.9 mg/kg/h IV for the following 30 hours. Infusion was discontinued when conversion was achieved or when any side effects were observed. Four out of 6 horses could successfully be converted to sinus rhythm but one of these horses showed symptoms of hind limb weakness and weight shifting. The two other horses that did not convert to sinus rhythm showed similar side effects. However, side effects disappeared completely in all the horses within 6 hours after termination of the AD infusion.

Clinical relevance:

Because of the acceptable success rate and non-cardiac side effects, a pharmacological study was warranted to evaluate the pharmacokinetics and bioavailability of AD in normal healthy horses.

In **Chapter 5** the clinical effects and pharmacokinetics of AD after single doses of AD were studied in a cross – over study. Six healthy adult horses received a single dose of 5 mg/kg of AD intravenously or orally. No adverse clinical signs were observed. This study revealed values of elimination half-lives (median: 51.1 hours for AD and 75.3 hours for desethylamiodarone [DAD]), clearance (0.35 (L/h)/kg), apparent volume of distribution (31.1 L/kg) and peak plasma concentrations of DAD (0.08 µg/mL after 2.7 hours) after the intravenous bolus. After the oral bolus, bioavailability (6.0- 33.7%), peak plasma for AD concentration (0.14 µg/ml attained after 7 hours) and for DAD concentration (0.03 µg/mL

attained after 8 hours) and elimination half-lives (median: 24.1 hours for AD and 58.6 hours for DAD) could be determined.

Clinical relevance:

The results of this study provide useful information for calculating an adapted AD treatment protocol for horses to achieve therapeutically plasma concentrations as described in human medicine.

In **Chapter 6** the adapted treatment protocol of intravenous AD (Cordarone ®) was used in 6 horses with chronic AF. In the first phase of the protocol, horses received a loading dose of 6.52 mg/kg/h of AD IV during 1 hour followed by 1.1 mg/kg/h IV for 47 hours. In the second phase of the protocol, horses received a second loading dose of 3.74 mg/kg/h of AD IV during 1 hour followed by 1.31 mg/kg/h for 47 hours. Infusion was discontinued if conversion to sinus rhythm was achieved or if side effects were observed. Three of the six horses could be cardioverted successfully without side effects. In 3 horses adverse effects of diarrhoea occurred and no cardioversion occurred in these patients.

Clinical relevance:

With this protocol the expected concentrations of AD and DAD amiodarone were achieved but the success rate was less than with the first described protocol. Adverse effects did only appear in the horses that received an AD treatment for more than 36 hours. These findings confirm the complexity of the AD molecule.

Indeed, as in human medicine, good results are obtained with electrical conversion. However, because cardioversion catheters to perform transvenous electrical cardioversion in horses were not available yet, a custom made catheter was constructed.

In **Chapter 7**, the transvenous electrical cardioversion technique with custom made catheters is described. Eight horses with chronic AF were used. As in the initial stage of this study, one horse showed multiple atrial premature complexes immediately after successful electrical cardioversion resulting in rapid recurrence of AF, it was decided to treat all subsequent horses with AD prior to the cardioversion procedure.

Six out of the 8 horses could successfully be converted to sinus rhythm, without obvious side-effects.

Clinical relevance:

Transvenous electrical cardioversion with custom made cardioversion electrodes can be considered as a treatment option for atrial fibrillation, especially when conventional drugs fail.

Pre-treatment with an anti-arrhythmic drug might help to reduce the risk of immediate recurrence of AF.

General conclusion:

In conclusion, horses suffering from short-term AF show rapid electrical and contractile remodeling and reverse remodeling after successful cardioversion suggesting that only a short rest period after successful cardioversion before returning to training is necessary. Horses suffering from long-term AF show rapid electrical and contractile remodeling, a slower increase in atrial dimensions and a slow reverse remodeling after successful cardioversion suggesting that a longer rest period after successful cardioversion is advisable before returning to training.

Propafenone, at a dose of 2 mg/kg over 15 minutes followed by a continuous infusion of 7 µg/kg/min during 85 minutes, is not efficient in horses with chronic AF. Intravenous AD has a moderate success rate and cumulative dosing (> 36 hours) may lead to adverse drug effects.

Transvenous electrical cardioversion is a potential alternative to pharmacological treatment. The combination of electrical cardioversion with anti-arrhythmic drugs might lower the incident of immediate recurrence of AF.

Atrium fibrillatie (AF) of voorkamerfibrillatie is de klinisch meest belangrijke ritmestoornis bij paarden. Ook in de humane geneeskunde wordt deze aritmie erg belangrijk geacht en intens bestudeerd. Niettegenstaande de talrijke onderzoeken is er eigenlijk nog maar weinig informatie beschikbaar over de pathofysiologie van AF bij het paard. Er zijn studies over pathofysiologie van AF bij de mens, de hond, de geit en de pony maar tot op heden is er niets gekend over het verloop van acute en chronische AF bij paarden. Informatie hierover bij paarden zou daarom nuttig zijn voor de kennis van de pathofysiologie van deze aritmie bij deze diersoort.

Tot op heden is quinidine sulfaat (QS) het meest gebruikte en beschreven anti-aritmicum voor de behandeling van AF bij paarden. Maar, wegens de toename van andere en/of betere anti-aritmica voor de behandeling van AF bij de mens wordt QS in sommige landen moeilijk verkrijgbaar. Er is zeer veel informatie beschikbaar over deze alternatieve producten voor behandeling van AF bij de mens maar over de toepassing ervan bij paarden is weinig gekend.

In het eerste deel van de algemene introductie worden de theorieën betreffende de pathofysiologie van AF beschreven zoals bekend uit de humane geneeskunde. In het tweede deel worden de werkingsmechanismen van de verschillende klassen van anti-aritmica en de behandelingsmogelijkheden voor AF bij de mens en het paard beschreven.

In het onderzoeksgedeelte van dit doctoraat vindt men drie secties. In de **eerste sectie** (Hoofdstukken 1-2) beschrijven we de elektrofysiologische, contractiele en dimensionale veranderingen die optreden tijdens acute en chronische AF bij paarden. In de **tweede sectie** (Hoofdstukken 3-6) geven we een beschrijving van nieuwe farmacologische behandelingsmethodes voor paarden met chronische AF. De **derde sectie** (Hoofdstuk 7) beschrijft de resultaten van het gebruik van elektrische cardioversie voor het behandelen van AF bij het paard.

In het **eerste onderzoek** werden 6 paarden voorzien van een pacemaker en een neurostimulator. De neurostimulator werd verbonden met een elektrische draad (lead) in de voorkamer. De pacemaker werd verbonden met één lead in de voorkamer en één lead in de kamer. Met de neurostimulator was het mogelijk om via snelle lokale elektrische ontladingen AF te induceren en te onderhouden gedurende een relatief korte periode van 7 dagen. De pacemaker gaf ons de mogelijkheid om elektrofysiologische metingen (genoemd atriale en ventriculaire effectieve refractaire periodes) te verrichten t.h.v. de voorkamer en de kamer en dit voor en tijdens de AF-periode, en na herstel van het normale sinus ritme. Op dezelfde tijdstippen werden echocardiografische metingen (voorkamer dimensies en contractiliteit) uitgevoerd.

Bij twee paarden echter, leidde dit korte AF-protocol tot persisterende AF waardoor het noodzakelijk was om een farmacologische behandeling in te stellen om het normale sinus ritme te kunnen herstellen.

In al de paarden zagen we een zeer snelle elektrische en contractiele verandering (24 uur) optreden tijdens de AF-periode. Na het herstel van sinus ritme zagen we dat de elektrische en contractiele veranderingen snel reversiebel waren (48 uur). Er werden geen significante veranderingen opgemerkt t.h.v. de kamer en de voorkamer dimensies.

Klinisch belang:

Uit dit onderzoek kunnen we besluiten dat het aangewezen is om snel een behandeling in te stellen na het optreden van AF bij paarden wegens de snelle elektrische en contractiele veranderingen die ontstaan na AF initiatie. Daarnaast blijkt dat na een geslaagde cardioversie van een kort durende AF periode er slechts een korte rustperiode nodig is voor volledig herstel.

In het **tweede onderzoek** werden er 4 paarden, geïnstrumenteerd met dezelfde techniek als bij het acute protocol (hoofdstuk 1) gebruikt. Bij deze paarden werd er met de neurostimulator een langdurige AF periode (4 maanden) geïnduceerd en onderhouden. Wegens de aanwezigheid van de pacemaker kon men elektrofysiologische veranderingen voor en tijdens de AF-periode, en na herstel van het sinus ritme gaan opvolgen. Op dezelfde tijdstippen

werden er ook echografische veranderingen (voorkamer dimensies en contractiliteit) opgevolgd.

Bij alle paarden traden er snel elektrische en contractiele veranderingen (24 uur) op, er werd ook een tragere toename van de voorkamer dimensies gemeten. Na herstel van sinus ritme duurde het echter 2 maanden alvorens de elektrische, contractiele en dimensionale veranderingen terug genormaliseerd waren. Er werden geen significante veranderingen gezien t.h.v. de kamer.

Klinisch belang:

Uit dit onderzoek kan men besluiten dat het aangewezen is om een rust periode van een 2-tal maanden in acht te nemen na een succesvolle behandeling van chronische AF (4 maand) om volledige normalisatie en remodelering te bekomen.

In **hoofdstuk 3**, onderzochten we het gebruik van propafenone (Rhythmnorm®), een klasse I anti-aritmicum, voor de behandeling van chronische AF. Twee paarden met natuurlijke, chronische AF en vier paarden met chronisch geïnduceerde AF werden behandeld met een propafenone protocol zoals gebruikt bij de mens. Alle paarden kregen 2 mg/kg propafenone IV over 15 minuten. Indien 20 minuten na deze bolus AF nog aanwezig was, kregen de paarden een onderhoudsdosis van 7 µg/kg/min propafenone IV gedurende 85 minuten. Het infuus werd onderbroken indien er neveneffecten werden opgemerkt of wanneer conversie tot sinus ritme optrad.

Met dit protocol zagen we geen cardiale of andere ernstige neveneffecten, maar, ondanks een significante verlenging van de atriale fibrillatie cycluslengte, trad geen cardioversie op.

Klinisch belang:

Uit dit onderzoek kunnen we besluiten dat het huidige gebruikte intraveneuze propafenone protocol niet efficiënt lijkt voor de behandeling van paarden met chronische AF.

In het **vierde hoofdstuk** beschrijven we het gebruik van amiodarone (AD) (Cordarone®), een klasse III anti-aritmica, bij paarden met chronische AF. Zes paarden werden behandeld met een AD protocol zoals voorgesteld bij de mens. De paarden kregen een ladingsbolus van 5

mg/kg/h AD IV gedurende 1 uur gevolgd door een eerste onderhoudsdosis van 0.83 mg/kg/h IV gedurende 23 uur. Vervolgens werd een hogere onderhoudsdosis toegediend van 1.9 mg/kg/h AD IV gedurende de volgende 30 uur. De behandeling werd stopgezet indien er neveneffecten optraden of indien er herstel van het normale sinus ritme optrad.

Bij vier van de zes paarden trad er herstel van normaal sinus ritme op maar bij één van deze paarden merkten we een parese van de achterhand op. Bij de twee andere paarden kon herstel van sinus ritme niet bekomen worden en trad hetzelfde neveneffect op. Na het stopzetten van de intraveneuze behandeling verdwenen deze neveneffecten binnen de 6 uur.

Klinisch belang:

Uit dit onderzoek konden we besluiten dat AD eventueel kon gebruikt worden als een alternatieve behandelingsmethode maar dat het aangeraden was om onderzoek te verrichten naar een aangepast behandelingsprotocol aan de hand van de farmacokinetische gegevens van AD bij gezonde paarden.

In **Hoofdstuk 5** onderzochten we via een cross-over studie het klinische effect en de farmacokinetiek van AD na een eenmalige toediening bij gezonde paarden. Zes paarden kregen een éénmalige orale of een intraveneuze bolus van AD. Bij geen van de paarden werden er neveneffecten opgemerkt. De eliminatie halfwaarde tijd (mediaan: 51.1 uur voor AD en 75.3 uur voor desethylamiodarone, een actief afbraakproduct van AD), klaring (0.35 L/kg·h), volume distributie (31.1 L/kg) en piek plasma concentratie van desethylamiodarone (0.08 µg/ml na 2.7 uur) na een eenmalige intraveneuze toediening werden hieruit bekomen. Van de orale bolus bekwam men de biologische beschikbaarheid (6.0-33.7%), een piek plasma concentratie van AD (0.03 µg/ml na 8 uur) en een eliminatie halfwaarde tijd (mediaan: 24.1 uur voor AD en 58.6 uur voor desethylamiodarone).

Klinisch belang:

De resultaten van deze farmacokinetische studie gaven ons de mogelijkheid om een aangepast behandelingsprotocol voor AD te berekenen voor het paard. Dit nieuwe protocol werd uitgerekend om plasma concentraties te kunnen bereiken die van therapeutische waarde zijn in de humane geneeskunde.

In **Hoofdstuk 6** pasten we het berekend AD protocol toe bij 6 paarden met chronische AF.

Tijdens een eerste fase kregen de paarden een ladingsbolus van 6.52 mg/kg/h IV gedurende 1 uur gevolgd door een onderhoudsdosis van 1.1 mg/kg/h IV gedurende 47 uur. In een tweede fase werd er een tweede ladingsbolus van 3.74 mg/kg/h AD IV gedurende 1 uur gegeven gevolgd door een onderhoudsdosis van 1.31 mg/kg/h gedurende 47 uur. Het infuus werd onderbroken indien er neveneffecten optraden of normaal sinus ritme bekomen werd. In drie van de zes paarden was het mogelijk om normaal sinus ritme te herstellen maar in de andere drie paarden was dit niet mogelijk en trad er tijdelijk diarree op.

Klinisch belang:

Met dit aangepaste protocol was het mogelijk om de vooropgestelde plasma concentraties te bekomen. Het slaagpercentage was echter lager dan in het eerst gebruikte humane protocol. De neveneffecten traden enkel op indien langer dan 36 uur AD infuus gegeven werd.

In de humane geneeskunde wordt er meer en meer gebruik gemaakt van transveneuze elektrische cardioversie als behandelingsmethode voor AF. De cardioversie katheters die hiervoor gebruikt worden bij de mens kunnen echter niet gebruikt worden bij het paard.

In **Hoofdstuk 7** beschrijven we de techniek en de mogelijkheid van gebruik van aangepaste cardioversie katheters voor de transveneuze elektrische cardioversie van AF bij het paard. Aangezien 1 paard, vlak na succesvolle cardioversie meerdere atriale premature complexen vertoonde die leidden tot onmiddellijk recidief van AF, werden alle erop volgende paarden vóór de eigenlijke elektrische cardioversie gedurende 24 uur behandeld met amiodarone.

Met deze techniek werd bij zes van de acht paarden normaal sinus ritme hersteld zonder belangrijke neveneffecten.

Klinisch belang:

Transveneuze elektrische cardioversie met aangepaste cardioversie katheters is een goed alternatief voor de behandeling van voorkamer fibrillatie bij het paard. Een voorafgaande farmacologische behandeling lijkt aangewezen om het risico van onmiddellijk AF recidief te minimaliseren.

Algemeen besluit:

Paarden die behandeld worden na een kortdurende AF-periode vertonen snel elektrische en contractiele veranderingen. Herstel van deze veranderingen na succesvolle behandeling is snel. Dit impliceert dat paarden best zo snel mogelijk behandeld worden na het optreden van AF en dat na conversie van een kortdurende AF-periode er slechts een korte herstel periode noodzakelijk is.

Paarden met chronische AF vertonen snel elektrische en contractiele voorkamer veranderingen en een tragere toename van de voorkamer dimensies. Na herstel van sinus ritme na een langdurige AF-periode wordt er aangeraden om een relatief lange herstel periode in acht te nemen zodat alle voorkamer parameters terug kunnen normaliseren.

Propafenone aan een dosis van 2 mg/kg gedurende 15 minuten gevolgd door een onderhoudsdosis van 7 µg/kg/min gedurende 85 minuten, lijkt niet efficiënt voor het behandelen van paarden met chronische AF.

De behandeling van paarden met intraveneuze AD heeft een matig slaagpercentage en een aanhoudende toediening (> 36 uur) kan leiden tot neveneffecten.

Men kan opteren voor interne elektrische conversie als alternatieve behandelingsmethode voor paarden met AF. Daarbij wordt er aangeraden om een farmacologische voorbehandeling te geven om het risico op direct herval van AF te verminderen.

CURRICULUM VITAE

Dominique De Clercq werd geboren op 27 januari 1974 te Gent. Na het behalen van het diploma Latijn-Wetenschappen aan het Koninklijk Atheneum te Blankenberge vervolgde ze haar studies voor het behalen van het diploma Diergeneeskunde, cum laude, op 2 juli 1999 aan de Universiteit Gent.

Onmiddellijk daarna trad zij in dienst bij de vakgroep Inwendige Ziekten en Klinische Biologie van de Grote Huisdieren. Ze nam deel aan de kliniekdiensten en gaf klinisch onderricht aan de studenten van de laatste jaren. Ze kreeg een algemene opleiding in de inwendige ziekten maar had extra interesse voor de klinische cardiologie van het paard. Ze droeg dan al een steentje bij aan het voorafgaande cardiologie werk dat verwerkt werd tot het doctoraat van Prof. Dr. G. van Loon. Door dat ze ook besmet was door het cardology virus van het paard en dankzij het voorafgaande doctoraatswerk, rolde ze zelf in het onderzoek in diezelfde tak. De resultaten van haar onderzoek werden samengebundeld en leidden uiteindelijk tot het doctoraatswerk “Pathophysiology and treatment of atrial fibrillation in horses”.

Dominique De Clercq is auteur of medeauteur van 17 publicaties in internationale en nationale tijdschriften. Ze nam ook actief deel aan 5 nationale en 13 internationale congressen. In april 2007 won ze de “Interpolis Award” voor de orale presentatie “Amiodarone and electrical cardioversion for atrial fibrillation” en in april 2008 won ze de “BEVA Award” voor de orale presentatie “Propafenone efficacy in horses with chronic atrial fibrillation”. Ze is ook reviewer voor internationale tijdschriften.

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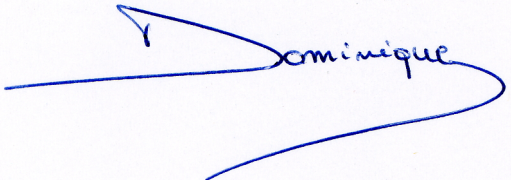
Ik wil ook het Bijzonder Onderzoeksfonds van de Universiteit van Gent bedanken voor hun financiële bijdrage van dit werk.

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Dominique