Department of Internal Medicine

University of Veterinary Medicine Budapest

Cilostazol as medical treatment for bradycardia of cardiac origin in dogs

Cilostazol hatóanyag vizsgálata a lassú szívveréssel járó ritmuszavarok kezelésére kutyákban

By

Ms. Silje Mørk

Supervisors:

Dr. Kiss Gergely and Dr. Manczur Ferenc

Budapest Hungary

2022

Abstract

Severe, pathological bradycardia is an arrhythmic condition where the heart rate is below the normal reference therefore the sufficient blood pressure is not upheld, resulting in symptoms such as weakness and syncope. In dogs, common pathological causes for bradycardia are sinus node dysfunction and AV blocks. These conditions usually require pacemaker as treatment. Pacemaker implantation is a surgery requiring high competence, expensive equipment and patients are often of old age and the summation of these factors causes a smaller motivation for the owner to proceed with the implantation. This results in the need for a medication more easily applicable and lower in cost.

Cilostazol is a drug originally intended for human patients with symptoms of intermittent claudation in peripheral vascular disease. It is a phosphodiesterase type 3 inhibitor, where it inhibits platelet aggregation and having a vasodilating effect by increasing the amount of protein kinase A. As a side effect it produces tachycardia. In some studies, it has shown promising effect in patients presenting with pathological bradycardia, both in humans and in dogs, where the use of the drug have increased and stabilized the heart rate. Despite the clinical success of cilostazol in several human studies, there are very few data (individual cases only) in animals that oral medication can substitute the need for pacemaker implantation or provide less symptoms in the period between diagnosis of bradycardia and scheduled pacemaker implantation.

The purpose of this prospective clinical study is to determine the safety, tolerability and clinical effects of cilostazol in severely bradycardic dogs due to cardiac disease. Six dogs ranging from 12-15 years (averaging at 14 years of age) was presented at the clinic with signs associated with bradycardia, all 6 showed signs of weakness and in addition 4 were presented with syncope. 3 dogs were diagnosed with sick sinus syndrome, 1 with 2nd degree AV block Mobitz II and 2 with 3rd degree AV block, with the using of ECG as diagnostic tool. Cilostazol was presented for the owners as an alternative drug, without knowing the full- or long-term effect. The dogs were given 10mg/kg Cilostazol b.i.d. orally. Follow up were made in frame of regular cardiological examinations. Data were evaluated using descriptive statistics and Student's t-test. All dogs showed increase in heartrate after administration of the drug averaging of 68% significant increase of baseline ($p=0,03$) and became asymptomatic with no side effects. The average symptom free survival time was 14 months (ranging 5-22 months). Even though our study population size is limited, we conclude that cilostazol is clinically effective, safe and well

tolerated in bradycardia of cardiac origin in dogs with AV block and sick sinus syndrome and may provide an alternative medical option to pacemaker implantation.

Összefoglalás

A súlyos, patológiás bradycardia olyan arrhythmia, amelyben a szívfrekvencia a normál referenciaérték alatt van. Ilyenkor nem megfelelő a vérnyomás, ami súlyos esetben gyengeséget és ájulást vagy akár hirtelen szívhalált is okozhat. Kutyákban a bradycardia gyakori kóros okai a sinuscsomó-diszfunkció és az AV-blokkok. Ezek az állapotok általában pacemaker beültetést igényelnek kezelésként. A pacemaker beültetés speciális szaktudást és költséges eszközöket igénylő műtét, a betegek gyakran idősek és ezért az állatok tulajdonosai nem minden esetben kérik ezt a beavatkozást. Mindez megteremti az igényét annak, hogy a bradyarrhythmiában szenvedő kutyáknak más alternatív, kevésbé költséges, gyógyszeres kezelést próbáljunk biztosítani.

A cilostazol egy olyan gyógyszer, amelyet eredetileg perifériás érbetegségben szenvedő embereknek szántak claudatiós tüneteik enyhítésére. A hatóanyag egy 3-as típusú foszfodiészteráz inhibitor, ahol a protein kináz-A mennyiségének növelésével gátolja a vérlemezke aggregátiot és tágítja az ereket. Mellékhatásként tachycardiát okoz. Egyes vizsgálatokban ígéretes hatást mutatott kóros bradycardiában szenvedő emberekben, illetve kutyákban, ahol a gyógyszer alkalmazása növelte és stabilizálta a szívfrekvenciát. Annak ellenére, hogy a cilostazol számos humán vizsgálatban sikeres volt, nagyon kevés adat áll rendelkezésre (és csak egyedi esetekben) állatokon arra vonatkozóan, hogy az orális gyógyszeres kezelés helyettesítheti-e a pacemaker beültetés szükségességét, vagy mérsékelheti a tüneteket a bradycardia diagnózisa és a tervezett pacemaker beültetés közötti időszakban.

Az általunk végzett prospektív klinikai vizsgálatnak a célja a cilostazol biztonságosságának, tolerálhatóságának és klinikai hatásainak tanulmányozása szívbetegség miatt súlyosan bradycardiás kutyákon. Az ÁTE beteganyagából származó hat, 12-15 év közötti (átlag 14 év) kutyát vizsgáltunk cardialis eredetű bradycardiával összefüggő tünetekkel, mind a hatnál gyengeség jelei mutatkoztak, ezenkívül négy kutya el is ájult. Három kutyánál sick sinus szindrómát, egy egyednél II-fokú Mobitz II-es és kettő kutyában III-fokú AV-blokkot diagnosztizáltunk EKG segítségével. A cilostazolt a pacemaker beültetés alternatív, gyógyszeres lehetőségeként említettük a tulajdonosoknak és elmondtuk, hogy nem ismerjük a gyógyszer teljes vagy hosszú távú hatását. A kutyák napi 10 mg/kg cilostazolt kaptak kétszer szájon át. A nyomon követés rendszeres kardiológiai vizsgálatok keretében történt. Az adatokat leíró statisztikák és Student-féle párosított t-próba segítségével értékeltük ki. Minden kutya a szívfrekvencia növekedését mutatta a gyógyszer beadása után, ami átlagosan 68%-os

szignifikáns növekedést jelent a kiindulási értékhez képest (p=0,03). Mellékhatásokat nem tapasztaltunk és az állatok tünetmentessé váltak. Az átlagos tünetmentes túlélés 14 hónap volt (5-22 hónap). Bár vizsgálati populációnk kicsi volt, jelen eredményeink alapján a cilostazol klinikailag hatékony, biztonságos és jól tolerálható AV-blokkban és sick sinus szindrómában szenvedő kutyáknál. Vizsgálatunk alapján a cilostazol gyógykezelési lehetőséget kínálhat a pacemaker beültetés helyettesítésére vagy a beültetésig történő klinikai stabilizációra.

TABLE OF CONTENTS

FIGURES AND TABLES

ABBREVIATIONS

ANP – Atrial Natriuretic Peptide

AP – Action Potential

AV – Atrioventricular node

BID – Bis in die “Two times a day”

BPM – Beats Per Minute

cAMP – Cyclic Adenosine Monophosphate

CNS – Central nervous system

ECG - Electrocardiogram

HR – Heart rate

LA – Left atrium

LL – Left leg

PDE - Phospodiesterase

PM - Pacemaker

PO – Per Os

PVC – Premature Ventricular Contraction

RA – Rigt atrium

SA – Sinoatrial node

SSS – Sick sinus syndrome

UCR – Urea Creatinine Ratio

US – Ultrasound

INTRODUCTION

Bradyarrhythmia is referring to when the heart is beating below its typical reference in a specific species. This can be due to problems sent out by the pacemaker cells in the heart, and/ or problems in the conduction of the heartbeat or due to extracardiac origin like electrolyte imbalance or increased parasympathetic activity. If the bradyarrhythmia is severe and is of cardiac origin a pacemaker might be installed, to pace how fast the heart is beating and create a stabile rhythm.

Many dogs, when diagnosed is not applicable for the pacemaker implantation. This can have several reasons; the origin of the bradyarrhythmia, the health of the animal, the economy of the owner or the procedure might not be offered in the region. A few studies, mainly done in Japan, have tested the use of cilostazol, as a therapeutic drug for bradyarrhythmia. Cilostazol is more commonly used in the human medicine field to treat intermittent claudation in peripheral vascular disorders.

As mentioned, there are only a few studies on the usage of the drug in dogs, however there are a few articles written on the use towards stabilizing the heart rhythm in humans, as an additional effect to its anticoagulative and vasodilating effect.

Finding a substitute or an alternative to the pacemaker implantation in the veterinary field is very relevant. The costs of the implantation, the surgery itself being invasive are very strong arguments for the owner to decline the implantation as treatment. The animal is then either euthanized or kept on medication to lessen the side effects of the disorder. The value of an oral tablet as opposed to a surgical treatment, would be very beneficial for all involved.

This clinical case study will deal with the hypothesis that cilostazol has the possibility of providing a medical therapy to lessen the symptoms of dogs with cardiac originated bradyarrhythmia.

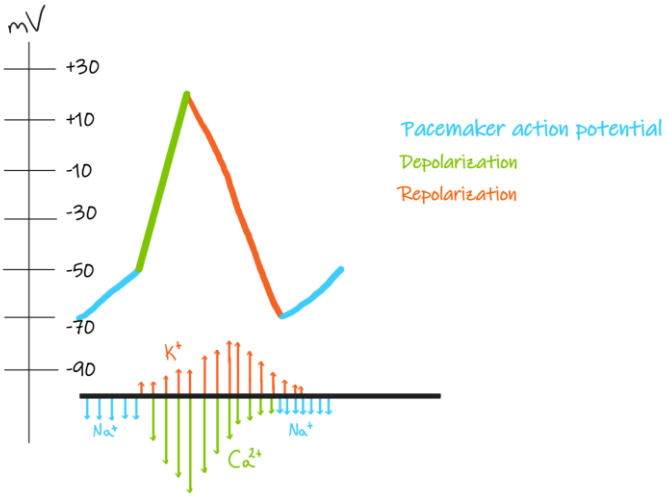
LITERATURE REVIEW:

1. THE CONDUCTION OF ELECTRICAL IMPULSES IN THE HEART AND HOW TO READ THEM

On three locations in the heart, specialized automatized myocardial cells, known as pacemaker cells, are creating electrical impulses without being influenced by other cells. These pacemaker cells make sure the heart have a rhythmic contraction to allow for the proper filling of blood into the heart and for the expulsion out of it. Pacemaker cells are found in the Sinoatrial (SA) node, which is located at the junction of the crista terminalis in the upper wall of the right atrium and the opening of the superior vena cava (Kashou, Basit and Chhabra, 2022). In the atrioventricular (AV) node which is located in the triangle of Koch, lying at the base of the right atrium (Kurian *et al.*, 2010) and in the Bundle of His, which is located distal to the atrioventricular node at the point where the AV node tissue enters the central fibrinous body, and connects the AV node with the Purkinje fibers (Patra, Zhang and Brady, 2022).

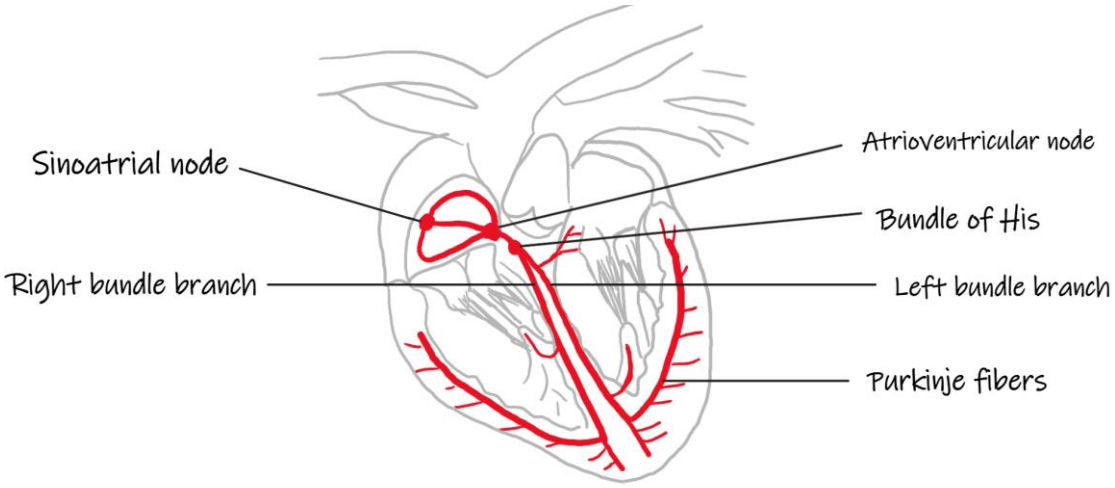
Under normal circumstances the electrical impulse originates in the SA node, created by the pacemaker cells located here. The cells will use the inflow and outflow of ions to create an action potential (AP). The cells depolarize by an influx of the positive charged Na^+ and Ca^{2+} by the help of voltage gated ion channels. These channels open and close when reaching different thresholds. Repolarizing starts when the Ca^{2+} gated channels close and K^+ voltage gated channels open. Followed by a depolarization again without a true resting potential. The flow of ions and the voltage which the pacemaker cells operate by can be seen in the left drawing of figure 1.

FIGURE 1 Depiction of the action potential in the pacemaker cells and in the non-pacemaking cells of the myocardium with the illustration of the electrolyte flow



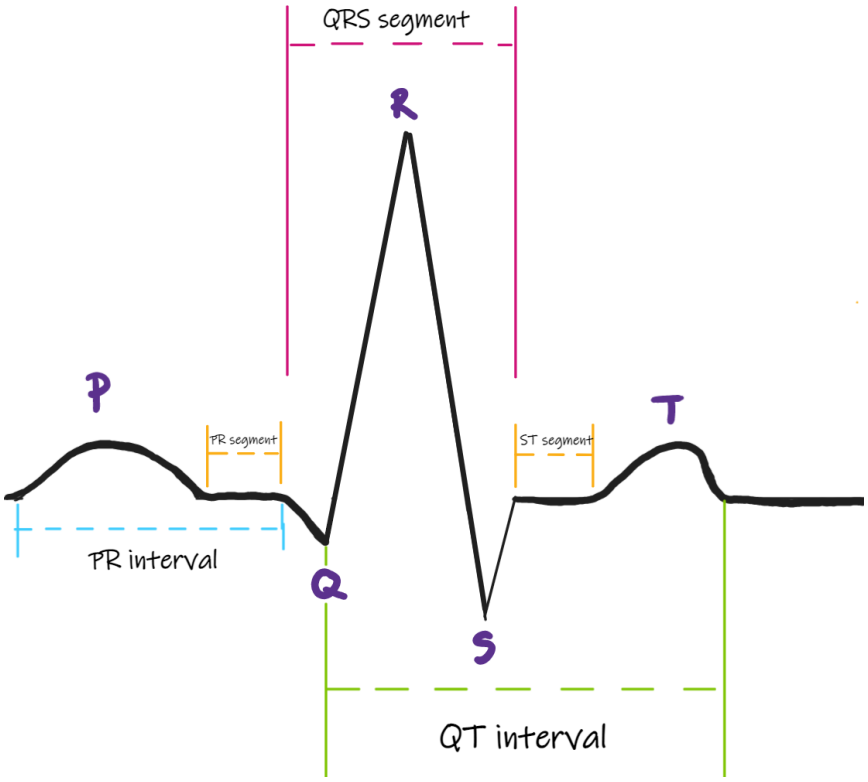
The fact that they are automatized and not having a resting potential, makes them different from the other non-pacemaker cardiomyocytes. This depolarization and repolarization occur around every second, giving the 60 beats per minute as a "normal" pace. The electrical impulse created by the pacemaker cells are then transmitted by the transitional cells to the right and left atrium, where the right atria is depolarized by 0.04 seconds before the left (Massumi *et al.*, 1969). Further the action potential reaches the AV node, and at this location there is a delay to make sure the atria has pumped the blood into the ventricles before they contract. The atria and ventricles are separated by a ring of fibrous tissue which is a part of the fibrous skeleton of the heart. This ring makes the AV node the main conduction pathway between the atria and ventricles. Not only is the task of the AV node to direct the electrical impulse and to delay the conduction, but it also limits the number of AP's conducted from atria to ventricle. In the case of increased firing from the atria, i.e., atrial fibrillation and it will be able to substitute in the case of SA dysfunction and lack of firing, as a backup system. After the SA node, the action potential is conducted further to the Bundle of His, down to the left and right bundle branches which is located within the septa down towards the apex of the heart and out into the network of the Purkinje fibers. The conduction through the Purkinje fibers transmits the AP evenly to create a simultaneous contraction in both ventricles (Assadi, 2016).

FIGURE 2 THE CARDIAC DEPOLARIZATION ROUTE.



The electrical impulses can be examined with the use of electrocardiography (ECG). The electrical impulses which are conducted in the heart can be measured on the surface of the skin, with the help of electrodes. The most used set-up for the leads is called Einthoven-lead system which contains three bipolar leads. Placed at the right arm/forelimb (RA), left arm/ forelimb (LA), and left leg/hindlimb (LL). They are also known as Lead I, where the RA is negative pole, and the LA is a positive pole. Lead II is travelling towards the positive pole on LL and finally the lead III which travels from the negative pole on the LA toward the positive LL. When depolarization occurs, a wave of positive charge is detected on the ECG. If the depolarization happen towards a positive electrode a positive deflection will be shown on the ECG. The limb leads register the heart electric activity in the horizontal plane in animals. If the wave of depolarization occurs away from the positive electrode, a negative deflection will be on the ECG. By the setup of leads and the systematical conduction of the heartbeat, the ECG shows a typical reading on the graph shown in figure 3 (Ware A. W., Ward L.J., 2020).

FIGURE 3 THE HEARTBEAT SHOWN ON THE ECG



These different deflections have been given letters for identification, to identify the conduction of the heart. The P is a smaller positive deflection showing the atrial depolarization. PR interval tells the time between the start of the P wave and the first deflection of the QRS complex. The QRS consists of three waves which represents the ventricular depolarization, where the Q is the depolarization of the interventricular septum, R wave is the main deflection of the ventricles and is shown as the biggest positive deflection on the ECG, S wave is the depolarization at the apex of the heart. The ST segment shows the period of zero potential between ventricular depolarization and repolarization. The T wave shows ventricular repolarization. These points on the ECG are used to interpret the rate and rhythm of the heart. To determine the instantaneous rate at which the heart is beating, one can count the squares (mms) between two adjacent QRS complexes and dividing this number into 3000 will yield the heart rate, because one minute is 3000 mm at 50 mm/s paper speed. This method is only valid in case of regular heart activity. The other method is to convert a certain length of the paper to seconds by knowing the paper speed and count the QRS complexes during that time period. For example, 6 second is 30 cm at 50mm/s paper speed, thus one can count all the QRS complexes in the 30 cm long record and multiply the number by ten will yield the average heart rate (beat/min). Latter is more accurate in the case of irregular rhythms. To establish the sinus rhythm, the P wave should be identified.

If every P wave is followed by a QRS and every QRS is preceded by P wave that indicates sinus origin rhythm. (Jackson, 2011)

2. BRADYARRHYTHMIA OF CARDIAC ORIGIN

Bradycardia refers to the slower than normal conduction of heartbeat, where the normal heartbeat for a medium sized dog is 100-160/min. A typical bradycardic dog will be presenting with a heartbeat slower than 60 beats per minute. This phenomenon can be present physiologically in very athletic dogs, in dogs treated with medication affecting the vagal tone (i.e., xylazine, medetomidine, digoxin), CNS trauma, hypothermia, hyperkalemia and in other diseases like hypothyroidism. Ware A. W., Ward L.J., (2020) describes well the different bradyarrhythmia of cardiac origin:

Dogs with **sinus bradycardia** will normally not show clinical signs that are associated with a slower heartbeat, and the heart rate will increase when physical activity is induced. In the case of a dog having a bradyarrhythmia resulting in symptoms, the heart rate will stay below 50 beats per minute. The symptoms that accompany bradyarrhythmia is weakness, exercise intolerance and syncope. (Ware A. W., Ward L.J., 2020).

Sick sinus syndrome (SSS) is when bradyarrhythmia occur due to abnormal function of the SA node. Presenting as a pause on the ECG. Here the typical signs as described with bradyarrhythmia are present. Miniature Schnauzer and West Highland White Terriers are breeds that are most affected, but other breeds also can be presenters of the syndrome. SSS can be present with other conductivity system problems which can be seen on the ECG. Worsening the clinical signs, and even seizures, these can be more like the behavior of metabolic or neurological seizures. (Ware A. W., Ward L.J., 2020).

Atrial standstill is a type of bradyarrhythmia, where there is no conduction from the atria, and junctional or ventricular escape beats are responsible for conducting the heart into a beat. This is a rare disorder and English Springer Spaniel being the overrepresented breed carrying this disease. Atrial standstill is often accompanied by inflammatory disorders of the myocardium. A more common form of atrial standstill is observed during severe hyperkalaemia. (Ware A. W., Ward L.J., 2020).

Atrioventricular conduction block, is when the atrium is not able to conduct to the ventricle to perform a regular beat. There are 3 types of AV blocks. In 1st degree AV block there is a delay of the impulse conduction in the AV node region, in an ECG the P wave is present, the

P-R interval is prolonged, but constant. In a 2nd degree AV block, some P waves are not followed by a QRS segment. We distinguish 2nd degree AV block on whether it is a Mobitz type I or type II. The difference between these types, is that in Mobitz type I the P-R interval will gradually lengthen until a QRS segment is not showing, and a beat is not conducted. In Mobitz type II, there is a constant interval between the P-R even when a conducted beat is missing. In 3rd degree AV block, there is no communication between the firing of the atrium and the ventricle. The QRS will only be formed by an escape beat and not on the instruction of the SA node. (Ware A. W., Ward L.J., 2020).

2.2 Diagnosing Bradycardia

Dogs are not often diagnosed with bradyarrhythmia, though its prevalence might be more common. A study done on beagles (Ulloa et al.) where 27 out of 44 males and 18 out of 46 females bradycardia was observed during 24-hour Holter-ECG records, in what seemed to be clinically normal dogs.

This underdiagnosis is probably since sinus bradycardia is usually asymptomatic. If clinical signs are present, the most common manifestations are syncope, shortness of breath and cyanosis. These are more easily observed by the owner and gives a cause for them to get their pet examined.

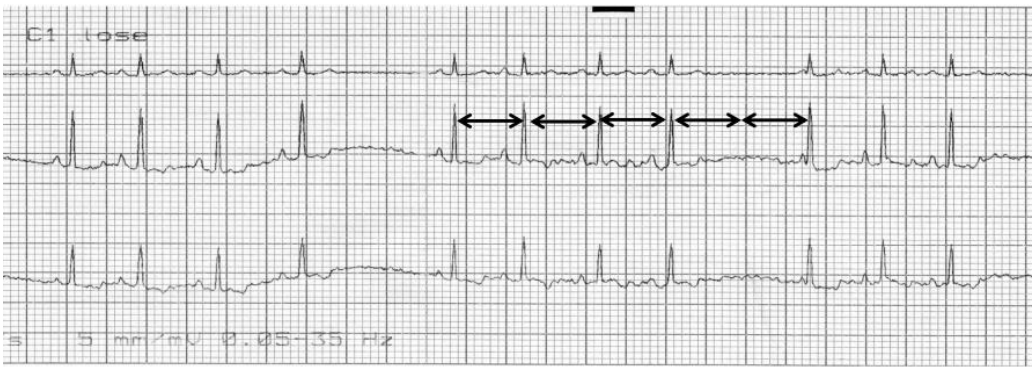
Preferred diagnostic tool is the **Electrocardiography** (**ECG)**, and with this tool we can visualize the electrical impulses conducted by the myocardial cells on the body surface of the animal (Kittleson, 2015). The electrodes are placed in the Einthoven- lead system on the right front limb (elbow), left front limb (elbow) the left hind limb (knee area). Other leads can be attached as well for further information like precordial/chest leads which can be used for the Holter ECG. The Holter ECG allows for registration of the heart rate in 24-48 hours.

The speed on the ECG is measured in mm/sec and is an adjustable feature (5, 25, 50 and 100 mm/sec) which must be considered when interpreting the heart rate. Sensitivity (voltage) of the leads are measured in mm/mV – higher sensitivity gives more detailed wave formation on the graph (2.5, 5, 10, 20 mm/mV).

Looking at the ECG the rhythm is regular, every P wave is conducted to a QRS in the case of a true sinus bradycardia. In the case of the rhythm being irregular, and there is a sinus bradycardia, it is referred to as respiratory arrhythmia. This due to increased vagal tone.

The blocks can be sinoatrial (SA) meaning the conduction from the sinus node is blocked or atrioventricular (AV) where the conduction of the impulse from the atria to the ventricles are blocked. SA blocks can be 1st, 2nd and 3rd degree blocks. In the case of SA blocks only 2nd degree can be diagnosed while 3rd degree cannot be differentiated from sinus arrest. 2nd degree SA block will appear in the ECG with one or more beats missing, and with a pause measuring exactly two PP distances, albeit sometimes more (Ware A. W., Ward L.J., 2020).

FIGURE 4 SHOWING 2ND DEGREE SA BLOCK



The **atropine challenge test** is a good diagnostic tool to evaluate the origin of the bradyarrhythmia, whether it be cardiac, extracardiac or differentiate it from the pronounced sinus arrhythmia (Rishniw M ; Tobias AH ; Slinker BK. 1996). When a dog is presenting with bradyarrhythmia, it is often with syncope as a main clinical sign. The dog is given atropine to challenge the hearts ability to increase the heart rate. Atropine is an anticholinergic drug which increases the sinus node rate and the AV conduction. It is a competitive muscarinic receptor antagonist. The atropine challenge test helps in determining if the bradyarrhythmia is caused by sinus and/or AV node dysfunction. It will decrease the vagal tone to confirm that the bradycardia is not due to the parasympathetic activity, if there is no effect the cause is cardiac. In case there is a real effect the cause is neurogenic, and the cause should be treated.

According to Ware A. W., Ward L.J., (2020) 0.04 mg/kg atropine is given IV, within 5-10 minutes the animal should be connected to ECG and if the results show a heart rate increased by 150% it is concluded not to be a “true” bradyarrhythmia. If the heart rate is not increased by 150% the test is repeated after 20 minutes. The purpose of administering atropine is that it will have no effect on a bradycardic heart where the reason for the slow conduction is an intrinsic disease of SA or AV node. It is important to mention however, that a portion of dogs with primary sinus node diseases also shows appropriate response during atropine test

2.3 THE CONVENTIONAL TREATMENT OF BRADYARRHYTHMIA

In the case of a positive atropine challenge test, atropine is used to treat the symptoms, and further diagnostics should be done to determine the cause of the bradyarrhythmia. If the atropine challenge is ineffective, further treatment with drugs like beta-agonists such as salbutamol and terbutaline or methylxanthine derivates like theophylline, aminophylline can be tried, but they have very limited effect if any. If the animal is not responding to medication, the only way to cure the bradyarrhythmia is by implanting a pacemaker (Gompf, 2011). Pacemaker implantation is done on a regular basis in the veterinary field, but is not always available, affordable or the patients' health does not indicate a safe procedure.

3. THE PHARMACOLOGY OF THE DRUG: CILOSTAZOL

Cilostazol is a drug more commonly used in the human medicine. It is used as an antithrombotic, vasodilator, antimitogenic and cardiotonic drug in human patients with peripheral artery disease and to treat the symptoms of intermittent claudication (Hiatt et al, 2008).

Cilostazol is a phosphodiesterase (PDE) 3A inhibitor. The task of PDA 3 is to increase breakdown of cAMP. cAMP or cyclic adenosine monophosphate, is involved in many biological reactions. It acts as a second messenger which gets stimulated by extracellular first messengers such as hormones and drugs.

In the heart the cAMP will affect protein kinase A which has a direct effect on Ca^{2+} channels which lead to increased calcium uptake and increased calcium release causing increased contractility (calcium induced calcium release), inotropic effect, chronotropic and dromotropic (conduction velocity) effect. The task of the phosphodiesterase is to break cAMP down to AMP. So, by inhibiting the phosphodiesterase with cilostazol more cAMP will be present, increasing the heart rate.

In the vessels the increased amount of cAMP will inhibit the myosin light chain kinase, therefor enzyme is responsible for causing contraction by inhibiting the myosin of the smooth muscles. This will lead to relaxation of the smooth muscles of the vessels. (Boularan and Gales, 2015)

The effect on the coagulation cascade is also regulated through cAMP, the task of cAMP is to decrease the cytosolic calcium levels. The task of the calcium is to release granules which will then activate more platelets and with it the coagulation cascade. (Rao, 2016)

Regarding the pharmacokinetics, cilostazol has a half-life of 10h, suggesting a b.i.d. administration. During the studies of Fukushima et al. (2018) a study was conducted to appropriate the dosage of cilostazol in dogs. In the studies 30 healthy beagles were given cilostazol in 2.5mg/kg, 5mg/kg or 10mg/kg, twice a day at 8 am and 8 pm in 10 days. During the studies it was concluded that the heart rate of the beagle dogs was significantly increased by the 5mg/kg and 10 mg/kg dosages. The R-R intervals were decreased at these dosages, and no higher frequencies of premature ventricular contractions were observed.

4. CILOSTAZOL IN HUMAN PATIENTS

Cilostazol showed in clinical use, first medical effect in the sinus node of humans, and it has been in use for 32 years in Japan and for the last 21 years in the United States. Over the course of the application of the drugs to treat arterial occlusive disease it has shown to have a positive chronotropic effect in patient with simultaneously occurring sick sinus syndrome, which means the heart will beat faster when the drug is administered. At the same time as it increases the heart rate, it will not, like other chronotropic drugs such as atropine or dopamine (Tisdale et al, 1995), cause an increase in AV blocks. In a case reported by Nimura et al (2011). An 83-year-old woman presenting with general fatigue was diagnosed with bradycardia and referred to undergo further ECG examination. During the exam advanced AV blocks was diagnosed with occasionally conducted beats. She was treated with a temporary pacemaker and due to coronary calcifications, she was given oral cilostazol at 200 mg. After 5 days of treatment, her heartrate had stabilized with a rhythm of 100 beats/min, and the temporary pacemaker was removed. After the pacemaker was removed whilst still on the cilostazol her heartrate was measured to be tachycardic at 110 beats/min. The conclusion is that the cilostazol treated the AV block, causing the bradycardic heart to become normalized towards tachycardic. In addition, a retrospective study was done to test the efficacy of cilostazol for SSS to avoid permanent pacemaker implantation. This study was performed by Sonoura et al, (2019) on 192 patients diagnosed with SSS, where 52 was treated with cilostazol and the rest served as a control group. The main focus was to study the PM implantation rate after 6 months. Secondary endpoint was the PM implantation rates after 1 and 3 months, HR after 1 week, 4 weeks and 6 months, longest sinus arrest interval and the side effects of the drug. The implantation rate was lower in the cilostazol group after 1,3 and 6 months.

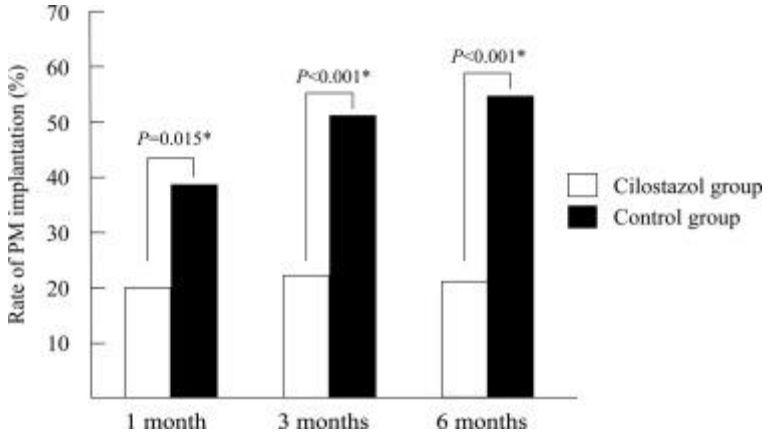


FIGURE 5 PERMANENT PACEMAKER (PM) IMPLANTATION RATES. THE CILOSTAZOL GROUP AND CONTROL GROUP SIGNIFICANTLY DIFFERED AFTER 1 MONTH (20.0% VS. 39.1%, RESPECTIVELY, P = 0.015), 3 MONTHS (20.8% VS. 50.4%, RESPECTIVELY, P < 0.001), AND 6 MONTHS (20.4% VS. 54) (SONOURA *ET AL.*, 2019)

The baseline heartrate was lower in the cilostazol group, indicating the severity of the disease in the patients. The HR was however increased in the three set points of time, making the study unbiased towards the baseline HR and at the same time shows the efficacy of cilostazol in increasing the heartrate. There was no increase in symptoms, no side effects or sudden death in the cilostazol group. The study concludes with cilostazol effect is decreasing the PM implantation by increasing the HR and doing it safely and efficiently in treating human patients with solidary bradycardic disease.

5. THE EFFECT OF CILOSTAZOL IN BRADYCARDIC DOGS – SSS

A report was written by Kanno and Szuki (2017), 6 years after Nimura et al (2011) report. They tested the function of cilostazol in a dog with sick sinus syndrome. A nine-year old intact male miniature schnauzer with syncope and unstable gait as symptoms was presented to the clinic. The clinical examination showed a grade 2/6 systolic murmur, irregular rhythm, and heart rate from 66-91 beat per min. X-ray showed an enlarged heart, confirmed as left ventricular hypertrophy with echocardiogram, along with mild mitral regurgitation, sinus arrest and AV block. ECG showed bradycardia and sinus arrest. The atropine test was performed without any result. With Holter PVCs and AV blocks were discovered leading to a diagnosis of severe sick sinus syndrome. Due to what is most often interest and/or economic reasons the decision from the owner was to not precede with a pacemaker implantation and standard treatment was implemented (isoprenaline and dipyridamole). The medication showed no improvement. After 7 days of treatment, the dog had elevated plasma atrial natriuretic peptide (ANP), and because of this was placed on cilostazol for 14 days. The dosage was 10 mg/kg twice daily PO. After these 2 weeks the owner reported less incidences of syncope and the ANP

had decreased, and after doing an examination with Holter, less PVC than previously observed was noted. The dog remained stable on cilostazol until it died of unknown cause 1,418 days after the treatment started.

A dog with these symptoms, not responding to the standard medical treatment should require a pacemaker. In this case a long-term therapy with cilostazol proved efficient in providing a good life quality and the writers suggest that cilostazol can be a future therapeutic agent for SSS.

EXPERIMENTAL SECTION

AIMS OF THE STUDY

The goal of this work was to study the safety, tolerability and clinical efficacy of cilostazol in a prospective pilot study in dogs with symptomatic, cardiac originated bradycardia.

MATERIAL AND METHODS

The examinations were carried out at the Department and Clinic of Internal Medicine at the University of Veterinary Medicine Budapest, between January 2021 and August 2022. A total of 6 dogs were presented to the clinic where cardiac originated symptomatic bradycardia was diagnosed. The dogs were applicable to this study as the pacemaker implantation was rejected by the owner. They were all castrated males. The average age was 14 years (range: 8-15). They have been through physical exams, total bloodwork including heartworm antigen-test, electrolytes, ECG, echocardiography and atropine challenge. The dogs were all followed up by regular cardiological control examinations.

Atropine challenge was performed to differentiate the true cardiac and the extracardiac causes for the observed bradyarrhythmia on the ECG. In the atropine challenge 0.04 mg/kg atropine was injected intravenously or subcutaneously and then a repeat of the ECG was performed after 15 or 30 minutes depending on the route of administration.

The ECG and echocardiography were performed by the university's internal medicine department under manual restraint, with no sedation. The animal was placed in right lateral recumbency on an echotable, with limbs straightened out. For the ECG examination a Schiller AT-1 3 channel and AT-2 plus 6 channels machine was used. The limb leads were placed in the initial examination according to the six standard limb leads (I, II, III, aVR, aVL and aVF). Alligator clip electrodes were attached to the unclipped skinfolds at LA, RA and LL. Contact was assured with the use of alcohol spray to the skin and alligator clips. The paper speed was

set to 25mm/s or 50 mm/s with a 5 or 10mm/mV amplitude and a total of 20-40 seconds was printed.

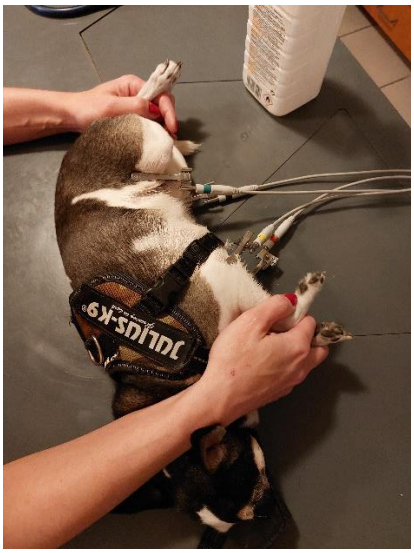


FIGURE 6 PATIENT 6 PLACED IN LATERAL RECUMBENCY FOR ECG MONITORING

For detailed echocardiography a Mindray DC-80A machine was used, with a phased array probe at conventional standard planes. A single Einthoven II-lead monitoring ECG was used during the whole echocardiographic process; thus, the heart rhythm was further monitored for another 10-20 minutes. Data analysis was performed using descriptive statistics and Student's paired t-test.

RESULTS

All six dogs included our study showed clinical signs related to bradycardia which was confirmed to be of cardiac origin at the clinic of the university. The individual patients age, breed and weight along with the diagnosis and symptoms made at the University of Veterinary Medicine Budapest's clinic, can be seen in table 1.

TABLE 1 DETAILED DATA OF THE STUDY POPULATION

Dog	Age	Breed	Weight (kg)	Diagnosis	Symptoms at first presentation
1	12	Cairn Terrier	9.2	Sinus node disfunction with regular sinus pause	Weakness, syncope
2	15	West Highland White Terrier	10.5	Sick sinus syndrome	Syncope
3	15	Bichon Frise	6.6	Sick sinus syndrome	Weakness
4	12	Yorkshire Terrier	8.0	2nd degree AV-block Mobitz II	Weakness
5	15	Beagle	15.0	3rd degree AV block	Weakness, syncope
6	8	Chihuahua	3.0	3rd degree AV block	Weakness, syncope

The cilostazol was well tolerated in a 10 mg/kg oral dose BID without side effects. Four of the six patients (66.67%) are still alive, living asymptomatic life, as detailed in Table 2.

TABLE 2 STATUS AND SURVIVAL OF THE PATIENTS AFTER CILOSTAZOL TREATMENT

Dog	Symptoms after cilostazol	Side effects	Treatment time (months)	Status
1	Asymptomatic	None	12	Alive
2	Asymptomatic	None	20	Alive
3	Asymptomatic	None	22	Alive
4	Asymptomatic	None	9	Dead
5	Asymptomatic	None	5	Dead
6	Asymptomatic	None	17	Alive

The cilostazol increased the heart rate in each individual case, but at different levels (Table 3). In this study population, the heart rate had increased at an average of 68% of baseline and in 50% of the patients sinus rhythm was seen. In the other 50% the heart rate was increased, but

AV blocks was still recorded. The effect on heart rate was significant between the baseline heart rates and the heart rates after cilostazol treatment; p=0.03 by the paired t-test. We found no relationship between the baseline heart rates and the change caused by cilostazol. Rather than simply increasing the heart rate of patients, it became apparent that apart of direct effects on the sinus nodal frequency, the number of extrasystoles (escape rhythm frequency, junctional and supraventricular extrasystoles) were also increased. The HR before and after treatment, the change in % of the baseline HR and rhythm can be seen in table 3 and in figure 7. No apparent trend is observed by the scatter plot seen in figure 8 in the baseline value and heart rate.

TABLE 3 EFFECT OF CILOSTAZOL ON THE HEART RATE (HR) AND RHYTHM OF THE INDIVIDUAL PATIENTS AND THE ENTIRE STUDY POPULATION

Dog	**HR at diagnosis (1/min)**	**HR after treatment (1/min)**	**HR change (% of baseline)**	**Rhythm after cilostazol**
1	80	140	75	Sinus rhythm
2	50	75	50	Sinus with occasional pause (3 sec)
3	60	150	150	Sinus rhythm
4	65	80	23	2nd degree AV block
5	35	70	100	3rd degree AV block with junctional extrasystoles
6	45	50	11	No change – 2nd degree AV block
Average	56	94	68	
SD	15	35	44	
Paired t-test of HR data before and after treatment: p=0,03-significant effect of treatment at p=0,05 level				

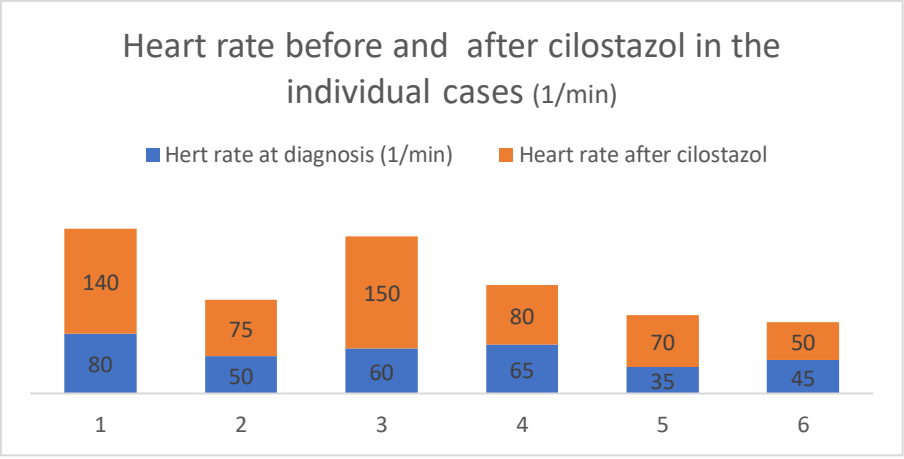


FIGURE 7 ILLUSTRATION OF HEART RATE CHANGES INDUCED BY CILOSTAZOL IN THE INDIVIDUAL CASES

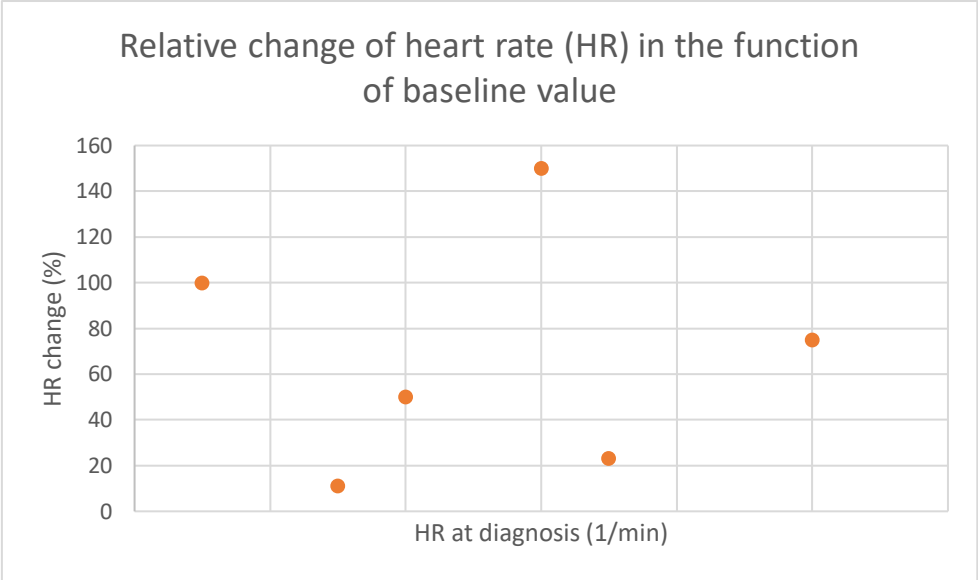


FIGURE 8 ILLUSTRATION OF THE LACK OF RELATIONSHIP BETWEEN HEART RATE CHANGES INDUCED BY CILOSTAZOL AND BASELINE HEART RATES

Patient number 6's ECG can be seen in the figure 9. The ECG to the left was taken at the first appointment with speed at 25mm/s and 10mm/mV showing the heartrate to be 45 bpm and in the ECG to the right with a speed of 50mm/s the HR 54bpm.

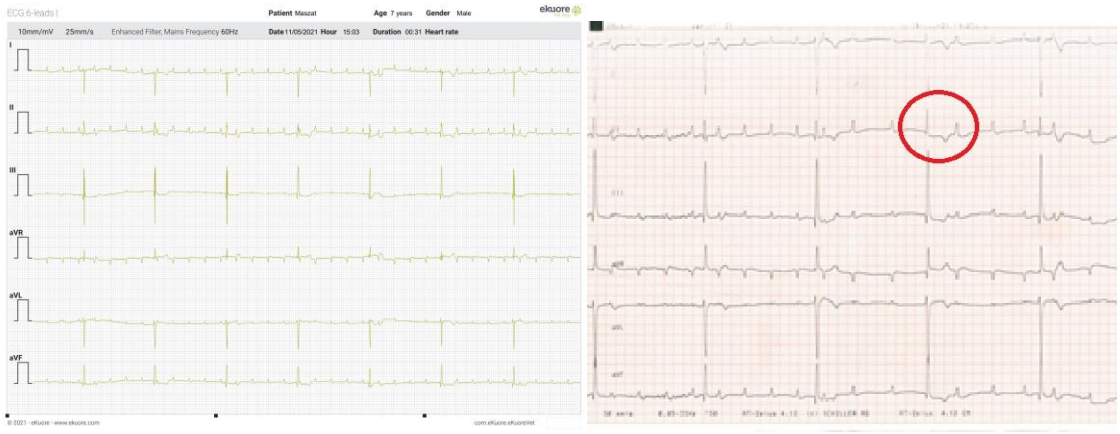


FIGURE 9 ECG BEFORE (45 BPM) AND AFTER CILOSTAZOL ADMINISTRATION (54 BPM). THE RHYTHM IS 3 RD DEGREE AV-BLOCK WITH JUNCTIONAL ESCAPE BEATS (RED CIRCLE)

To prove the effect of the drug, a withdrawal was performed on patient 3. This patient was clinically stable and had initially only mild symptoms thus withdrawal was without serious concerns and by the owner's preference (to avoid unnecessary medication). 3 months after the start and stabilization of the patient due the effect of the cilostazol, the medication was withdrawn to investigate if the HR had by itself stabilized to relieve the patient from its symptoms or if it was in fact the effect of the drug. In the following figures (Figures 11-14) the changes in the ECG seen on the ultrasound machine and HR which was registered by the built in ECG of the ultrasound machine.

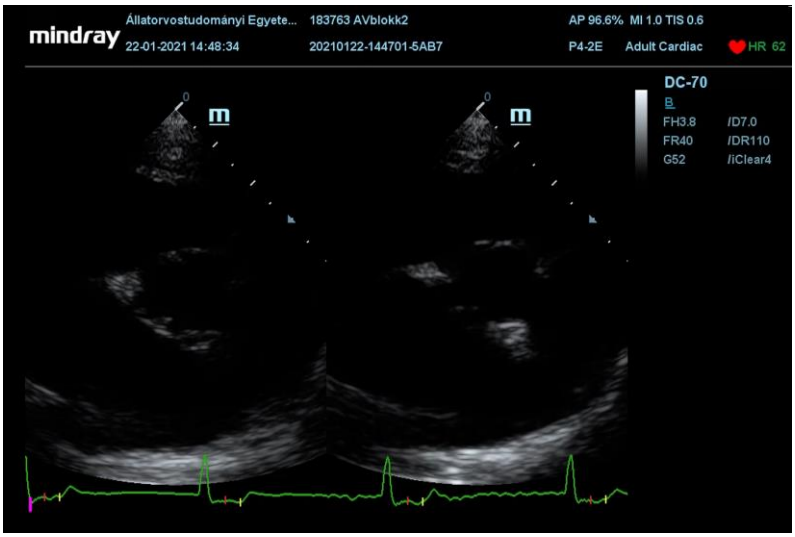


FIGURE 10 PATIENT 3 AT FIRST PRESENTATION. PLEASE NOTE THE LACK OF P-WAVES AND THE SLOW HEART RATE ON THE ECG MONITOR.

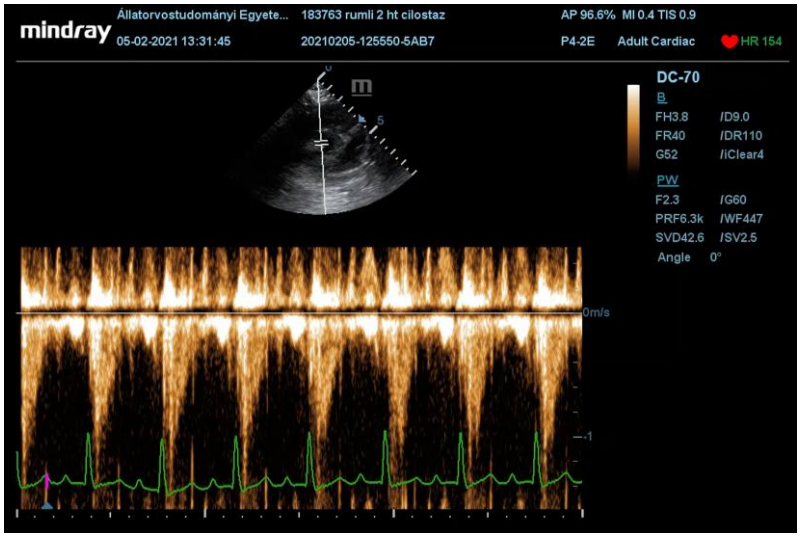


FIGURE 11 CHECK-UP CONTROL OF PATIENT 3 AFTER HAVING RECIEVED CILOSTAZOL. PLEASE NOTE THE SINUS RHYTHM AND HIGHER HEART RATE ON THE ECG MONITOR

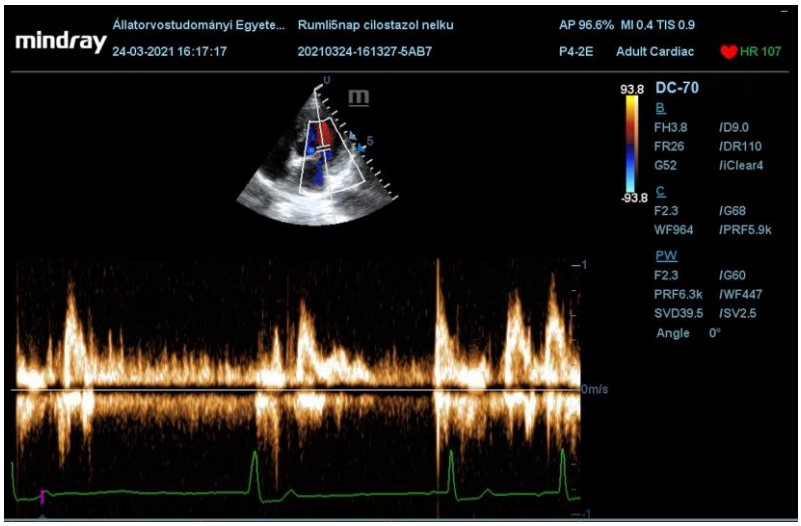


FIGURE 12 THE PATIENT 3 HAD BEEN WITHDRAWN FROM THE MEDICATION FOR 5 DAYS. PLEASE NOTE AGAIN THE SLOW ESCAPE RHYTHM ON THE ECG MONITOR.

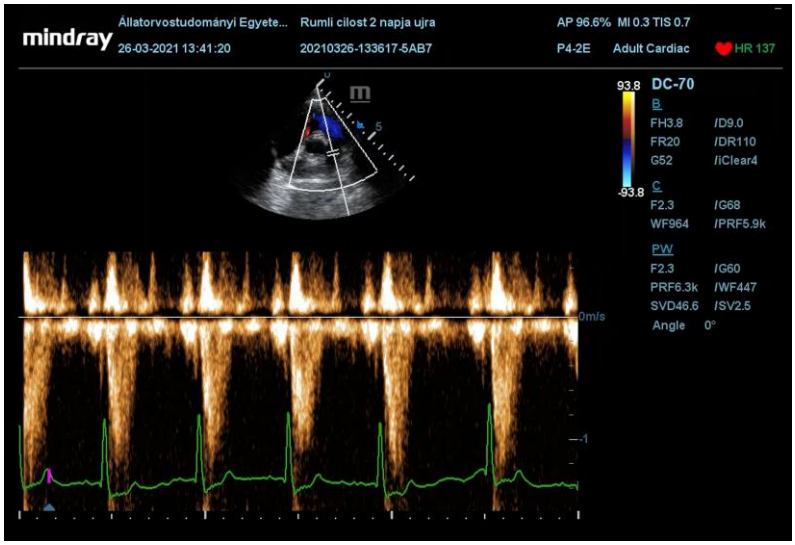


FIGURE 13 SAME PATIENT (PATIENT 3) STARTED ON CILOSTAZOL AGAIN

The HR was initially at 62 with regular sinus pauses (sick sinus syndrome), increasing to 154 on cilostazol and showing normal sinus rhythm, reducing to 70 after withdrawal with ECG signs of sick sinus syndrome and increased up again at 137 after re-administration of the drug 48 hours later showed sinus rhythm again, suggesting direct drug effect.

DISCUSSION

The cilostazol was well tolerated in all dogs at a 10 mg/kg oral dose BID without side effects. At the time of writing this thesis 66.67% of the patients are still alive, living asymptomatic life. The average survival time so far is 15 months, but since patients are still alive an exact survival time cannot be determined.

As for the two patients who did not survive other problems complicated the condition of the patient. Patient 4 was suffering from chronic kidney disease and severe proteinuria (UCR: 5,7) with concurrent AV conduction disease (2nd degree AV block) and responded well to cilostazol for 9 months until a transient 3rd degree block developed and metabolic acidosis (pH 7,2) was diagnosed. Acidosis was probably related to decompensation of kidney failure which might had been related to transient thrombosis in the kidneys. The patients improved after the treatment of acidosis. After 4 days the phenomenon was repeated with severe weakness and 3rd degree AV block, and the patient died spontaneously. This death was probably not related to cilostazol, but the lack of effect may have been related to extracardiac cause of bradycardia, which overwhelmed cilostazol's medical potential. Patient 5 was an absolute candidate for pacemaker implantation because of severe hemodynamic instability due to a 3rd degree AV-block of cardiac origin. After living almost asymptomatically (mild weakness) with good quality of life on cilostazol treatment for 5 months, the 15 years old patient died suddenly during sleep. This death cannot be directly linked to the use of cilostazol, but more probably reflect the natural course of severe bradyarrhythmic disease.

With a drug withdrawal trial on patient 3, it was clearly demonstrated the reversible effect of cilostazol on increasing the heart rate and influencing the cardiac rhythm. This also means that the effect the drug is only symptomatic and not curative. Mechanism of action is not clear and cilostazol only induces tachycardia in certain forms, sinus or supraventricular tachycardia, including junctional tachycardia. The increased heart rate was a long-term effect resulting in significant hemodynamical and clinical improvement and a much better quality of life in our study population. However, our study is strongly limited by the low number of cases, and we only have data from limited number of diseases (AV-block and sick sinus syndrome patients). Another limitation of our study was the lack of Holter-ECG exam of our patients. Even though my supervisors made all effort to monitor the heart rhythm during the entire exam period (20-30 minutes) this still only reflected maximum of 2% of the entire day's heart activity. Further Holter-ECG studies are needed to more reliably judge the therapeutic potential of this new

medical therapy. Nevertheless. our results showed that cilostazol may be a promising medical alternative of pacemaker implantation or clinical stabilization of severe symptomatic bradyarrhythmia before the pacemaker implantation. Naturally, more data are needed from a higher number and a more diverse set of patients (e.g. myocarditis which is a major inflammatory cause of severe, transient AV-block).

CONCLUSION

This pilot clinical study, even though with a limited sample size, showed together with the literature reviewed, that cilostazol is an effective and well tolerated drug in dogs. Cilostazol was effective in 100% of patients if only patients with normal laboratory findings were included. Based on our findings cilostazol may be effective in cases where the bradycardia is of cardiac origin (AV block, SSS) and in these patients can be used as an alternative to pacemaker implantation. Cilostazol may also be a drug of importance in stabilizing the hemodynamically unstable patient prior to pacemaker implantation.

BIBLIOGRAPHY:

'The ECG Leads, Polarity and Einthoven's Triangle' (2015) *The Student Physiologist*, 20 October. Available at: https://thephysiologist.org/study-materials/the-ecg-leads-polarity-and-einthovens-triangle/ (Accessed: 8 October 2022).

Ashley, E.A. and Niebauer, J. (2004) *Conquering the ECG, Cardiology Explained*. Remedica. Available at: https://www.ncbi.nlm.nih.gov/books/NBK2214/ (Accessed: 8 October 2022).

Assadi. 2016 'Conduction System of the Heart: Overview, Gross Anatomy, Natural Variants'. Available at: https://emedicine.medscape.com/article/1922987-overview (Accessed: 8 October 2022).

Boularan, C. and Gales, C. (2015) 'Cardiac cAMP: production, hydrolysis, modulation and detection', Frontiers in Pharmacology, 6, p. 203. Available at: https://doi.org/10.3389/fphar.2015.00203.

Cardiopulmonary Resuscitation in Animals - Emergency Medicine and Critical Care (no date) *MSD Veterinary Manual*. Available at: https://www.msdvetmanual.com/emergency-medicine-and-critical-care/specific-diagnostics-and-therapy/cardiopulmonary-resuscitation-in-animals?query=atropine%20bradycardia (Accessed: 21 March 2022).

CV Pharmacology | Phosphodiesterase Inhibitors. Available at: https://www.cvpharmacology.com/vasodilator/PDEI (Accessed: 21 March 2022).

Determining Rate. Available at: https://www.healio.com/cardiology/learn-the-heart/ecg-review/ecg-interpretation-tutorial/determining-rate (Accessed: 8 October 2022).

Diagnosis of Heart Disease - Circulatory System (no date) *MSD Veterinary Manual*. Available at:https://www.msdvetmanual.com/circulatory-system/heart-disease-and-heart-failure/diagnosis-of-heart-disease (Accessed: 19 April 2022).

François, L. *et al.* (2004) '[Pacemaker implantation in dogs: results of the last 30 years]', *Schweizer Archiv Fur Tierheilkunde*, 146(7), pp. 335–344. doi:10.1024/0036-7281.146.7.335.

Fukushima, R. *et al.* (2018) 'Effects of cilostazol on the heart rate in healthy dogs', *Journal of Veterinary Medical Science*, 80(11), pp. 1707–1715. doi:10.1292/jvms.18-0240.

Hiatt, W.R., Money, S.R. and Brass, E.P. (2008) 'Long-term safety of cilostazol in patients with peripheral artery disease: the CASTLE study (Cilostazol: A Study in Long-term Effects)', *Journal of Vascular Surgery*, 47(2), pp. 330–336. doi:10.1016/j.jvs.2007.10.009.

Jackson, D.L.P. (2011) 'Understanding an ECG | ECG Interpretation | Geeky Medics', 5 March. Available at: https://geekymedics.com/understanding-an-ecg/ (Accessed: 12 October 2022).

Kanno, N. and Suzuki, T. (2017) 'Long term effects of cilostazol in a dog with sick sinus syndrome', *Journal of Veterinary Medical Science*, 79(6), pp. 1031–1034. doi:10.1292/jvms.17-0018.

Kashou, A.H., Basit, H. and Chhabra, L. (2022) 'Physiology, Sinoatrial Node', in *StatPearls*. Treasure Island (FL): StatPearls Publishing. Available at: http://www.ncbi.nlm.nih.gov/books/NBK459238/ (Accessed: 7 October 2022).

Kellum, H.B. and Stepien, R.L. (2006) 'Third-degree atrioventricular block in 21 cats (1997-2004)', *Journal of Veterinary Internal Medicine*, 20(1), pp. 97–103. Available at: https://doi.org/10.1892/0891-6640(2006)20[97:tabic]2.0.co;2.

Kittleson, 2015 'Diagnosis of Heart Disease - Circulatory System MSD Veterinary Manual. Available at: https://www.msdvetmanual.com/circulatory-system/heart-disease-and-heart-failure/diagnosis-of-heart-disease (Accessed: 12 October 2022).

Kurian, T. *et al.* (2010) 'ANATOMY AND ELECTROPHYSIOLOGY OF THE HUMAN AV NODE', *Pacing and clinical electrophysiology : PACE*, 33(6), pp. 754–762. Available at: https://doi.org/10.1111/j.1540-8159.2010.02699.x.

Massumi, R.A. *et al.* (1969) 'Time sequence of right and left atrial depolarization as a guide to the origin of the P waves', *The American Journal of Cardiology*, 24(1), pp. 28–36. Available at: https://doi.org/10.1016/0002-9149(69)90047-2.

Ware A. W., Ward L.J., 2020: Cardiovascular system disorders in: Nelson, R.W. and Couto, C.G. (eds.)2020: Small Animal Internal Medicine. Sixth edition. Missouri, Elsevier. P86-88, P35-37, P42-44

Nimura, A. *et al.* (2011) 'Recovery of Advanced Atrioventricular Block by Cilostazol', *Internal Medicine*, 50(18), pp. 1957–1961. doi:10.2169/internalmedicine.50.5228.

Patra, C., Zhang, X. and Brady, M.F. (2022) 'Physiology, Bundle of His', in *StatPearls*. Treasure Island (FL): StatPearls Publishing. Available at: http://www.ncbi.nlm.nih.gov/books/NBK531498/ (Accessed: 7 October 2022).

Rao GH. Role of cyclic AMP and cyclic GMP as modulators of platelet cytosolic calcium. J Clin Prev Cardiol 2016;5:99-103

Rebecca E. Gompf (2011) '*Sinus bradycardia (2011)'* by Saunders, an imprint of Elsevier Inc. Available at: https://www.saintfrancis.org/wp-content/uploads/Sinus-Bradycardia.pdf

Rishniw M ; Tobias AH ; Slinker BK. Characterization of chronotropic and dysrhythmogenic effects of atropine in dogs with bradycardia. Am J Vet Res 1996;57[3]:337-41

Sonoura, T. et al. (2019) 'Efficacy of cilostazol for sick sinus syndrome to avoid permanent pacemaker implantation: A retrospective case–control study', Journal of Cardiology, 74(4), pp. 328–332. Available at: https://doi.org/10.1016/j.jjcc.2019.03.007.

Tisdale, J.E. *et al.* (1995) 'Electrophysiologic and proarrhythmic effects of intravenous inotropic agents', *Progress in Cardiovascular Diseases*, 38(2), pp. 167–180. doi:10.1016/s0033-0620(05)80005-2.

Ulloa, H.M., Houston, B.J. and Altrogge, D.M. (1995) 'Arrhythmia prevalence during ambulatory electrocardiographic monitoring of beagles', *American Journal of Veterinary Research*, 56(3), pp. 275–281.

What is an electrocardiogram (ECG)? (2019) *InformedHealth.org [Internet]*. Institute for Quality and Efficiency in Health Care (IQWiG). Available at: https://www.ncbi.nlm.nih.gov/books/NBK536878/ (Accessed: 8 October 2022).

HuVetA
ELECTRONIC LICENSE AGREEMENT AND COPYRIGHT DECLARATION*

Name: Silje Mørk

Contact information (e-mail): Siljemrk95@gmail.com

Title of document (to be uploaded): Cilostazol as medical treatment of bradycardia of cardiac origin in dogs

Publication data of document: 17.10.2022

Number of files submitted: 1

By accepting the present agreement the author or copyright owner grants non-exclusive license to HuVetA over the above mentioned document (including its abstract) to be converted to copy protected PDF format without changing its content, in order to archive, reproduce, and make accessible under the conditions specified below.

The author agrees that HuVetA may store more than one copy (accessible only to HuVetA administrators) of the licensed document exclusively for purposes of secure storage and backup, if necessary.

You state that the submission is your original work, and that you have the right to grant the rights contained in this license. You also state that your submission does not, to the best of your knowledge, infringe upon anyone's copyright. If the document has parts which you are not the copyright owner of, you have to indicate that you have obtained unrestricted permission from the copyright owner to grant the rights required by this Agreement, and that any such third-party owned material is clearly identified and acknowledged within the text of the licensed document.

The copyright owner defines the scope of access to the document stored in HuVetA as follows (**mark the appropriate box with an X)**:

☒ I grant unlimited online access,

☐ I grant access only through the intranet (IP range) of the University of Veterinary Medicine,

☐ I grant access only on one dedicated computer at the FerencHutÿra Library,

☐ I grant unlimited online access only to the bibliographic data and abstract of the document.

Please, define the **in-house accessibility of the document** by marking the below box with an **X**:

☒

I grant in-house access (namely, reading the hard copy version of the document) at the Library.

If the preparation of the document to be uploaded was supported or sponsored by a firm or an organization, you also declare that you are entitled to sign the present Agreement concerning the document.

The operators of HuVetAdo not assume any legal liability or responsibility towards the author/copyright holder/organizations in case somebody uses the material legally uploaded to HuVetA in a way that is unlawful.

Date: Budapest, 17….day 10…….month…2022.year

Author/copyright owner signature Silje Mork

HuVetAMagyar Állatorvos-tudományiArchívum – Hungarian Veterinary Archive *is an online veterinary repository operated by theFerencHutÿraLibrary, Archives and Museum. It is an electronic knowledge base which aims to collect, organize, store documents regarding Hungarian veterinary science and history, and make them searchable and accessible in line with current legal requirements and regulations.*

HuVetA relies on the latest technology in order to provide easy searchability (by search engines, as well) and access to the full text document, whenever possible.
Based on the above, HuVetA aims to:

- *increase awareness of Hungarian veterinary science not only in Hungary, but also internationally;*
- *increase citation numbers of publications authored by Hungarian veterinarians, thus improve the impact factor of Hungarian veterinary journals;*
- *present the knowledge base of the University of Veterinary Medicine Budapest and its partners in a focussed way in order to improve the prestige of the Hungarian veterinary profession, and the competitiveness of the organizations in question;*
- *facilitate professional relations and collaboration;*
- *support open access.*