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THEMED ISSUE REVIEW

The canine chronic atrioventricular block model in cardiovascular preclinical drug research

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Ventricular cardiac arrhythmia is a life threating condition arising from abnormal functioning of many factors in concert. Animal models mirroring human electrophysiology are essential to predict and understand the rare pro- and anti-arrhythmic effects of drugs. This is very well accomplished by the canine chronic atrioventricular block (CAVB) model. Here we summarize canine models for cardiovascular research, and describe the development of the CAVB model from its beginning. Understanding of the structural, contractile and electrical remodelling processes following atrioventricular (AV) block provides insight in the many factors contributing to drug-induced arrhythmia. We also review all safety pharmacology studies, efficacy and mechanistic studies on anti-arrhythmic drugs in CAVB dogs. Finally, we compare pros and cons with other *in vivo* preclinical animal models. In view of the tremendous amount of data obtained over the last 100 years from the CAVB dog model, it can be considered as man's best friend in preclinical drug research.

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KEYWORDS
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1 | INTRODUCTION

Cardiovascular diseases are a major cause of death in the world. In this aspect, cardiac arrhythmias form an important quantitative and qualitative category (Al-Khatib et al., 2018). There are several types of cardiac arrhythmias, some of which are directly life-threatening, such as ventricular fibrillation, whereas others increase the risk of secondary conditions, such as atrial fibrillation (Al-Khatib et al., 2018). Although treatments of cardiac arrhythmias are still improving and expanding, such as device technology and catheter ablation, pharmacotherapy remains an important pillar in the clinic (Al-Khatib et al., 2018). Many anti-arrhythmics have been developed that can directly or indirectly inhibit a ventricular or atrial arrhythmia. Moreover, new pharmacological targets are still being discovered for which medication is being developed, although the amount of new anti-arrhythmics in different phases of development is still rather low (Camm, 2017), which definitively requires the united attention from academia and industry. Importantly, in each drug discovery and development trajectory, *in vivo* testing using animal systems is still necessary and mandatory by legislation (ICH Guideline S7B, 2005). Many *in*

Abbreviations: AV, atrioventricular; CAVB, chronic atrioventricular block; LQTS, long QT syndrome; SDR, spatial dispersion of repolarization; STV, short-term variability of repolarization duration.

silico and *in vitro* systems are currently available, or are being improved or developed as part of the Comprehensive *in Vitro* Proarrhythmia Assay (CiPA) initiative, that can evaluate one or more factors underlying cardiac arrhythmia. These systems are positioned mostly in the intermediate phases of drug development. Especially *in silico* action potential models combined with dynamic clamp approaches and/or human-induced pluripotent stem cell-derived cardiomyocytes as testing systems almost completely integrate the interplay of the ion channels and transporters that generate the human action potential and their collective responses to new chemical entities (Gintant et al., 2016). Thus far however, only *in vivo* systems can show the intricate interplay between cardiac electrical activity, pump function, haemodynamic feedback, neural regulation, electrolyte homeostasis, display of spatial dispersion of repolarization (SDR) and their collective response to pharmacological treatment in one model.

The ideal animal system for cardiac drug evaluation does not exist. But such a model should at best mirror human properties such as ion channel expression, both in type and interdependence, haemodynamics, neural control of the heart, pharmacodynamics, cardiac size and so forth. In other words, a model should include all biological aspects that are important or currently perceived to be important, for the occurrence of cardiac arrhythmia. With respect to these factors, the dog is considered a suitable model (Clauss et al., 2019).

Canines have been an important model for (electro) cardiac research since the start of experimental physiology in the 19th century. They have been used for example in research on sinus arrhythmia (Ludwig, 1847), the cardiac conduction system (Tawara, 1906), the ventricular excitation pattern (Durrer & Van der Tweel, 1957) and many more physiological mechanisms involved in the function of the heart.

Here, we will present an overview of different canine models used in preclinical cardiovascular research, followed by an in-depth exploration of one of the most frequently used canine models in cardiac safety pharmacology and anti-arrhythmic drug research, the chronic atrioventricular block (CAVB) dog model.

2 | CONCORDANCE BETWEEN HUMAN AND CANINE PHYSIOLOGY

To appreciate the extent to which the results of different cardiac disease models in dogs can be translated to the human situation, it is important to know the similarities and differences between the canine and human heart. First off anatomically, the heart sizes of large dogs are comparable with human heart sizes, but obviously, large dogs display a higher heart weight to body weight ratio. The canine coronary anatomy is different to the human situation, as there is an extensive collateral circulation and left dominant system in dogs. Electrophysiologically, dogs compare reasonably well with humans. The anatomy of the conduction system is quite similar, given the length of the His bundle, the distribution of the Purkinje fibres along the ventricular wall and the location of the atrioventricular (AV) node just above the tricuspid valve. Moreover, almost all major currents found in humans are present in dogs. These similarities explain the close resemblance between canine and human action potential waveform, both portraying typical notching (Clauss et al., 2019). However, the relative contributions of the ionic currents do vary considerably between humans and dogs. I_{K1} and I_{Ks} densities were found to be threefold and 4.5-fold larger in dogs than in humans, whereas I_{Kr} density was similar. As these are the major repolarizing currents, the redundancy in human repolarization is thus far smaller than in dogs. In other words, humans have a smaller repolarization reserve, 'safety reserve' to withstand challenges on the I_{Kr} current (Jost et al., 2013). We will further elaborate on the concept of repolarization reserve in Section 5. Because of the different composition of canine ionic currents, there are important electrophysiological differences between human and dog hearts. In sinus rhythm during rest, atrial and ventricular action potential duration, and therefore all the ECG parameters, are shorter in dogs than in humans (dogs vs. humans: P wave 40 ms vs. 110 ms, PR interval 60–130 ms vs. 120–200 ms, QRS duration 50–60 ms vs. 84–110 ms, QT interval 150–250 ms vs. 400–430 ms). Dogs also have a higher heart rate and respiratory rate than humans (dogs vs. humans: heart rate 80–160 vs. 60–80 bpm and 15–25 vs. 12–15 rpm). Since the QT interval physiologically shortens as the heart rate increases, it is very important to correct the QT interval appropriately when interpreting canine preclinical data. Several correction formulas have been designed based on large patient cohorts and applied in clinical practice, such as the Bazett, Fridericia and Framingham formulas. However, the Van de Water formula has been specifically designed to correct for QT intervals in anaesthetized dogs. Indeed, when applying different QT correction formulas on dog data, the Van de Water formula gives the best approximation (Patel et al., 2017). We therefore recommend the use of the Van de Water formula when correcting the QT interval in preclinical research in dogs.

The systolic blood pressure (BP) in dogs is higher than in humans, but diastolic BP is lower (136/66 in dogs vs. 120/80 mmHg in humans) (Clauss et al., 2019). A final physiological difference is that there is a more pronounced and more variable respiratory sinus arrhythmia in dogs, which results in a higher heart rate variability. Importantly, increased heart rate variability does not necessarily indicate that the canine heart is more susceptible to arrhythmias in general. In heart failure patients, for example, loss of respiratory sinus arrhythmia is often found and is associated with poor long-term outcomes, such as sudden cardiac death. The higher heart rate variability in dogs should thus be interpreted as a reflection of a different balance between cardiac sympathetic and parasympathetic innervation and thus a species-specific trait. Indeed, an increased parasympathetic tone to the canine heart is one of the major contributors to the elevated heart rate variability (Moïse et al., 2020). The autonomic nervous system (ANS) is a well described factor in the development of cardiac arrhythmias. Therefore, the different balance in sympathetic versus parasympathetic innervation between humans and dogs is an important factor to be considered when interpreting preclinical research in canine cardiac disease models (Fukuda et al., 2015).

Other than the increased parasympathetic tonus in the canine heart, the innervation of the human and canine heart are very similar.

Sympathetic innervation arises from the stellate ganglion, where preganglionic nerves activate postganglionic nerves by releasing the neurotransmitter **acetylcholine (ACh)**. The postganglionic sympathetic nerves exert their effect on the heart by releasing **noradrenaline.** The parasympathetic cardiac innervation originates mainly from the nucleus ambiguus, located in the medulla of the brain. The preganglionic, cranial nerve that projects from the nucleus ambiguus to the postganglionic nerves is the vagal nerve or the 10th cranial nerve. ACh release, in case of the parasympathetic division, not only activates of the postganglionic nerves but also transmits the postganglionic nerve activity to the heart (Mizeres, 1955).

All in all, the dog has been described as the most predictive species in the cardiac electrophysiological research field, which underlines the consonance between canine and human cardiac electrophysiology. Other large animals with more comparable coronary anatomy, like pigs, have proven superior in myocardial ischaemia research (Clauss et al., 2019; Gralinski, 2003).

A second important factor to consider for translational validity of canine cardiac disease models, particularly cardiac drug evaluation, is pharmacokinetics. Compared with humans, dogs have some different characteristics that can alter the pharmacokinetics of drugs and therefore make extrapolation between canines and humans difficult. These characteristics include altered gastric pH, intestinal motility and permeability, plasma protein binding, physiological volumes and the substrate specificity of the **CYP450** enzymes, that is, the hepatic enzymes that perform catabolic reactions on compounds (better known as 'first-pass metabolism'). It is therefore very important to monitor plasma concentrations of administered compounds when investigating the effect of a drug in canine preclinical cardiac disease models (Tibbitts, 2003).

3 | CANINE MODELS IN CARDIOVASCULAR RESEARCH

Due to the aforementioned translational benefits that dogs pose for studying cardiac diseases, a plethora of models have been developed. Table 1 shows an overview of the different types of dog models that have been used in cardiac preclinical research and their effectivity. Please note that the selected references serve as illustrative examples and do not represent a comprehensive list of all studies that exploit the respective model. The table is divided in three major categories: electrical heart disease, heart failure and myocardial infarction. The first category, electrical heart diseases, is subdivided into ventricular and atrial arrhythmias. Notably, the majority of the models require electrical stimulation protocols to evoke episodes of arrhythmias and are therefore performed under anaesthesia. The second category, heart failure, is subdivided into models that approach heart failure with a reduced ejection fraction, the 'classic' form of heart failure and models that approach heart failure with a preserved ejection fraction. The latter category has recently gained increasing clinical interest, as subjects display typical symptoms of heart failure but do not demonstrate a loss of ejection fraction. They do however show impaired myocardial relaxation, hampering the filling of the heart during diastole and thereby contributing to the clinical symptoms of heart failure. As this is a relatively new insight in the field of heart failure, far fewer models for the latter subcategory have been described than for 'classic' heart failure. Within the subcategory of heart failure with a reduced ejection fraction, the models have been organized based on the pathological condition they simulate, that is, ischaemia, pressure/volume overload and a residual category. Finally, the last major category consists of myocardial infarction. This category displays methods to induce myocardial infarction in the context of developing (pharmacological) interventions to limit the size of the infarcted area. Notably, the induction of ischaemia is also listed under electrical heart disease and heart failure, but in these cases, the aim of these studies was to induce arrhythmias or heart failure, respectively. Therefore, the induction of ischaemia was instrumented to study other endpoints in these cases, whereas infarction size was the primary outcome in the 'myocardial infarction' category.

Table 1 shows more overlap, majorly between heart failure and electrical heart diseases, of the use of seemingly similar techniques being used to cause different disease models. The following will provide insight in this concordance and the development of different dog models over the decades.

The use of dog models to study cardiac diseases started early in the 20th century with the induction of atrial and ventricular fibrillation by electrical stimulation. Using several stimulation protocols, short episodes of fibrillation could be induced to study the electrical and mechanical properties of the heart during fibrillation (Brams & Katz, 1931; Eyster & Swarthout, 1920; Wiggers, 1930; Wiggers & Wegria, 1940). A decade later, the severity of the induced episodes was increased by adding acute myocardial infarction or sympathetic modulation to the stimulation protocols (Harris, 1950; Wiggers et al., 1940; Wilburne et al., 1947).

The ischaemic techniques were further developed over the years and came closer to the human pathological conditions that predispose for the development of arrhythmias. Myocardial infarction was applied more chronically, as coronary occlusion was approached less aggressively and was followed by a period of remodelling. Due to the aforementioned extensive collateral coronary circulation, chronic coronary occlusion alone was usually not sufficient to induce arrhythmias. Additional stimulation protocols or compounds remained necessary as an 'additional hit' (Damiano et al., 2015; El-Sherif et al., 1977; Nishida et al., 2011; Ohara et al., 2002; Sinno et al., 2003). Without these 'additional hits' as pacing, sympathetic modulation or drugs, different methods for causing cardiac ischaemia were applied in models of ischaemic cardiomyopathy (Feola et al., 1971; He et al., 2004; Munagala et al., 2005; Sabbah et al., 1991; Saku et al., 2018). The same goes for chronic atrioventricular block, mitral regurgitation, increased afterload and tachypacing-induced cardiomyopathy: on their own, these conditions were induced in the context of studying heart failure (Arita et al., 2007; Armstrong et al., 1986; Gaasch et al., 1989; Kleaveland et al., 1988; Sasayama et al., 1976; Starzl & Gaertner, 1955). In combination with electrical stimulation protocols or drugs, they served as models to study atrial or ventricular fibrillation in the context of a

TABLE 1 Overview of canine models for cardiac disease

Category	Model	Procedures	Effectivity	Reference
Electrical heart diseases				
Ventricular arrhythmias	Electrical stimulation protocol	Faradic stimulation (A)	Not reported	Wiggers, 1930
		Shock in vulnerable period (A)	Not reported	Wiggers & Wegria, 1940
	Sympathetic modulation	i.v. epinephrine + deep cyclopropane anaesthesia (A)	9/11 spont. VT paroxysms, varying from 11 to 302 s	Wilburne et al., 1947
	MI (acute/chronic)	Acute LAD ligation + chronic two-stage occlusion (A)	After acute ligation 3/4 VF; after two-stage occlusion 4/4 VT in large infarct size, 2/6 VT in small infarct size	Harris, 1950
	MI (acute/chronic) + (ESP/compounds)	Acute LAD dissection + shock in vulnerable period (A)	7/7 dogs VT/VF	Wiggers et al., 1940
		Double LAD ligation, after 3–7 days of premature stimulation (A)	40/45 VT (5/45 small infarction due to collaterals)	El-Sherif et al., 1977
		Transient LAD occlusion + pacing 80 bpm + compounds (A)	Flecainide 4/6 VT/VF, dofetilide 0/6	Damiano et al., 2015
	Chronic AV block + compounds	Formaldehyde injection ventricular septum, diuretics + compounds (A)	Quinidine 2/5 TdPs, propranolol 1/5 VT, sotalol 4/5 TdPs	Weissenburger et al., 1991
	Chronic ventricular tachypacing	VVI 250 bpm for 3–5 weeks	6/25 SCD (polymorphic VT), average 6.7 NSVT runs/dog/24 h	Pak et al., 1997
	Aortic constriction and insufficiency	Aortic leaflet perforation, 6 weeks later banding of abdominal aorta, then repeated 24-h Holter recordings	After 240 days 14/26 VT, after 720 days 25/26 VT	Zhu et al., 2014
	Kidney disease + ESP	Kidney artery ligation until 50%–60% kidney infarction, 6 weeks of remodelling + premature stimulation (A)	8/8 VF	Tang et al., 2017
	Long QT syndrome + sympathetic modulation	i.v. I_{Ks} blocker (HMR1556) + isoproterenol bolus (A)	17/18 TdPs	Gallacher et al., 2007
	Long QT syndrome + sympathetic modulation + ESP	i.v. caesium chloride + adrenaline + overdrive pacing (A)	8/8 VA (4/8 VT, 3/8 TdPs, 1/8 VF)	Levine et al., 1985
	Hereditary ARVC	Naturally occurring myocardial disease in boxer dogs	9/23 SCD, 19/23 ventricular arrhythmias, all dogs (fibro)fatty replacement (absent in control dogs)	Basso et al., 2004
Atrial arrhythmias	Electrical stimulation protocol	Faradic stimulation of the right auricle (A)	Flutter 15/?, fibrillation 7/?	Eyster & Swarthout, 1920
		Faradic stimulation of the right auricle (A)	Max. 25 s of auricular flutter/fibrillation	Brams & Katz, 1931
	Electrical parasympathetic stimulation + ESP	Bilateral cervical vagal trunk stimulation + rapid pacing bursts (A)	16/16 persistent (>30 min) atrial fibrillation, terminated when vagal stimulation stopped	Wang et al., 1992
	Chemical parasympathetic stimulation	ACh injection in pulmonary vein fatpad containing autonomic ganglia (+ESP)	2/5 spontaneous atrial fibrillation (AF), 3/5 susceptible to single extrastimulus. Avg. AF duration 9.6 min	Po et al., 2005

TABLE 1 (Continued)

Category	Model	Procedures	Effectivity	Reference
		Carbachol injection in pulmonary vein fatpad containing autonomic ganglia (+ESP)	2/6 spontaneous atrial fibrillation (AF), 4/6 susceptible to single extrastimulus. Avg. AF duration 37.7 min	Po et al., 2005
	Atrial lesion + ESP	Y-shaped intracaval lesion, after 2 weeks of rapid burst pacing	5/5 non-self-terminating flutter, variable susceptibility	Frame et al., 1986
		Crush injury on right atrial free wall + acute rapid and premature pacing (A)	8/8 dogs sustained (>10 min) flutter, variable susceptibility	Feld & Shahandeh-Rad, 1992
	Chronic ventricular tachypacing + ESP	3 weeks VVI 240, 2 weeks VVI 220 bpm, then burst pacing (A)	10/18 sustained (>30 min), 8/18 non-sustained atrial fibrillation	Li et al., 1999
	Chronic atrial tachypacing + ESP	Atrial pacing 400 bpm, after 6 weeks of premature stimulation/rapid burst if necessary (A)	18/22 sustained (>15 min) atrial fibrillation, 2/22 non-sustained, 2/22 non-inducible	Morillo et al., 1995
	Chronic atrial tachypacing + mitral regurgitation	Transection of chordae tendinae of mitral valve + atrial pacing 640 bpm, after 6 weeks 24 h of Holter recording	16/16 spontaneous persistent atrial fibrillation	Mitchell et al., 1997
	Mitral regurgitation + ESP	Transection of chordae tendinae of mitral valve, after 3 months of rapid burst pacing if necessary (A)	3/25 spontaneous fibrillation, 16/25 after burst pacing (5/16 non-self-terminating), 6/25 non-inducible	Cox et al., 1991
	Sterile pericarditis + ESP	Pericardiotomy, talcum powder on atrial surface, after recovery rapid premature pacing	23/25 flutter, 17/23 lasting >5 min	Pagé et al., 1986
	Chronic systemic inflammation + ESP	Peridontitis through silk ligatures around premolars, after 60/90 days of extrastimuli (A)	60 days: 5/12 fibrillation, duration avg. 1.55 s, 90 days: 10/12 fibrillation, duration avg. 4.76 s	Yu et al., 2010
	Ischaemic cardiomyopathy + ESP	Permanent LAD occlusion, after 6–8 weeks of burst pacing (A)	5/5 dogs inducible for atrial fibrillation, average 28 s	Ohara et al., 2002
	Atrial infarction (acute/chronic) + ESP	Double ligation of RIAA + acutely burst pacing (A)	9/20 dogs prolonged (>20 min) atrial fibrillation	Sinno et al., 2003
		Double ligation of RIAA, after 8 days of burst pacing (A)	All dogs showed atrial fibrillation, average duration increased from 30 s (sham) to 1146 s	Nishida et al., 2011
Heart failure				
Reduced ejection fraction				
Ischaemia (acute/chronic effect)	LAD ligation	LAD ligation + intermittent ligation of collaterals until 'medium-sized ischaemia'. Then 2–6 h of observation	2 h: LVp decreased from 136 to 98 mmHg, LVEDP increased from <12 to 15 mmHg, LVEF decreased from 68% to 27%; 6 h: 80% mortality due to VF	Feola et al., 1971

(Continues)

TABLE 1 (Continued)

Category	Model	Procedures	Effectivity	Reference
	LAD ligation – reperfusion	LAD + major diagonal branches ligation for 180 min, then reperfusion and 4 weeks of remodelling	LVEDP increased from 3.9 to 15.0 mmHg, d*P*/d*t* and LVEF decreased (2032 to 1982 mmHg and 64% to 47%)	Saku et al., 2018
	Sequential coronary microembolization	Every 1–3 weeks of injection of 3–6 ml of latex microspheres suspension in LAD/circumflex via implanted coronary catheter until LVEF < 35%. Then 3 months of remodelling	LVEF and CO decreased (64% to 21% and 2.9 to 2.3 $L \cdot min^{-1}$). LVEDP and LVEDV increased (6 to 22 mmHg and 64 to 101 ml), 30% in-study mortality, 26% atrial fibrillation	Sabbah et al., 1991
Pressure/volume overload	Chronic aortic constriction	Inflatable cuff around ascending aorta, repeated measurements of LV dimensions	After 19 days of LV wall thickness increased 15%, peak wall stress increased 22%	Sasayama et al., 1976
		Expanding band around ascending aorta of 8-week-old puppies, 12 months of follow-up	6/16 dogs clinical signs of heart failure, LV: body weight 9.8 $g \cdot kg^{-1}$, LVEDP 25 mmHg (compensated group: 7.7 $g \cdot kg^{-1}$ and 8 mmHg)	Gaasch et al., 1989
	Mitral regurgitation	Disruption of mitral chordae/leaflets until significant mitral regurgitation. Then 12–17 months of follow-up	16/22 dogs survived >3 months, 11/15 severe regurgitation (RF > 0.5). Severe group: LVEDV, LVEDP and LV mass increased (48–85 ml, 9–16 mmHg and 71–90 g), CO decreased (2.3 to 1.8 $L \cdot min^{-1}$)	Kleaveland et al., 1988
	Chronic AV block	Section of the region with the bundle of His; then 3 months of remodelling	5/11 congestive heart failure (hepatomegaly + pulmonary vascular congestion) in resting state, 7/11 under daily exercise, 1/7 dogs SCD	Starzl & Gaertner, 1955
Other	Chronic ventricular tachypacing	VVI 250 until a clear biological endpoint for heart failure	After avg. 5.3 weeks: >25% increase in cardiac size (X-ray) and/or >10% increase body weight	Armstrong et al., 1986
	Chronic mechanical dyssynchrony + ventricular tachypacing	His bundle ablation + VVI 170 for 4 weeks; left bundle branch ablation + atrial pacing 200 bpm for 6 weeks	9/9 dogs heart failure + broad QRS: LVEF decreased from 58.4% to 21.4%, EDV increased from 49.6 to 71.3 ml	Arita et al., 2007
	Cytotoxic agents (anthracyclines)	Weekly Adriamycin injection in the LAD for 5 weeks, then 3 weeks remodelling	6/6 clinical heart failure (1 required medication), LVEDV increased (76 to 99 ml), LVEF and CO declined (54% to 25% and 5.6 to 3.9 $L \cdot min^{-1}$)	Magovern et al., 1992
	Chagas cardiomyopathy	Intraperitoneal inoculation of the VL-10 strain of *Trypanosoma cruzi*, after 6–9 months + echocardiography (A)	28% of dogs LVEF < 40% (established cut-off for dilated cardiomyopathy in dogs)	Carvalho et al., 2019
	Hereditary dilating cardiomyopathy	Naturally occurring myocardial disease in Portuguese water dogs	Dogs died 2–32 weeks after birth, all post-mortem signs of dilating cardiomyopathy	Werner et al., 2008
Preserved ejection fraction	Sequential coronary microembolization	Daily coronary injection of microbead suspension until endpoint heart failure was	9/21 heart failure (LVEDP > 18 mmHg and signs) with preserved systolic function	He et al., 2004

TABLE 1 (Continued)

Category	Model	Procedures	Effectivity	Reference
		reached (LVEDP ≥ 18 mmHg). Then 2.5 weeks of remodelling	(dP/dt > 2650 mmHg), 6/21 systolic heart failure (LVEDP 23 mmHg, dP/dt < 2650 mmHg), 6/21 lesser degree of LVEDP rise	
	Chronic hypertension	Renal wrapping with cellophane; then 6–8 weeks of remodelling	Increased MAP (170 vs. 140 mmHg), impaired LV relaxation (53 vs. 35 ms), higher LV mass to body weight (5.5 vs. 4.9 $g \cdot kg^{-1}$), more fibrosis (3.4 vs. 2.0%), preserved ejection fraction (49% vs. 55%)	Munagala et al., 2005
Myocardial infarction				
Open chest	Surgical coronary artery ligation	Clamp of proximal circumflex artery for 3 h, then reperfusion and measurements; adjustable snare occluder around left circumflex artery until cyanosis of 75% of the inferior wall, 7 days of follow-up	3/14 SCD, infarct size 26% of LV; 4/24 SCD, infarct size 22% of LV	Reimer et al., 1985
	Adjustable constrictor	Adjustable constriction of LAD using ameroid constrictor + exercise during 14–21 days	Subendocardial blood flow decreased from 1.19 to 0.51 $ml \cdot min^{-1}$, systolic wall thickening decreased from 24.3% to 6.0%	Heusch et al., 1987
Closed chest	Embolization	Inject flexible plugs that swell after contact with fluids in a coronary artery, follow-up for 14–37 days	1/8 dogs died 2 days after procedure (infarct size 26% of LV), other infarctions varied from 2% to 21%, 6/8 infarctions transmural	Herr et al., 1985
		Induce coronary thrombosis by passing a current through an electrode in a major coronary branch until total occlusion; then max 16 days of follow-up	23/23 total occlusion of major branch, infarction size varied depending on competence of collateral circulation	Salazar, 1961

Note: Summarizes the different canine models used for cardiac preclinical research, how these models are induced and their effectivity. The selected references serve as illustrative examples and do not represent a comprehensive list of all studies that exploit the respective model.

Abbreviations:- (A), measurements under anaesthesia; ARVC, arrhythmogenic right ventricular cardiomyopathy; AV, atrioventricular; avg., average; CO, cardiac output; ESP, electrical stimulation protocol; LAD, left anterior descending coronary artery; LV, left ventricle; LVEDP, left ventricular end-diastolic pressure; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVP, left ventricular pressure; MAP, mean arterial pressure; MI, myocardial infarction; RF, regurgitation fraction; RIAA, right intermediate atrial artery; SCD, sudden cardiac death; spont., spontaneous; TdP, Torsade de Pointes; VA, ventricular arrhythmia; VF, ventricular fibrillation; VT, ventricular tachycardia; VVI, ventricular demand pacing.

remodelled heart (Cox et al., 1991; Li et al., 1999; Morillo et al., 1995; Weissenburger et al., 1991; Zhu et al., 2014). This introduction of 'co-pathology' is in line with clinical practice, as there is a strong association between heart failure and rhythm disturbances in patients (Al-Khatib et al., 2018).

Some heart failure-based models for electrical heart disease are also effective in producing spontaneous arrhythmias without the need for pacing protocols or drugs to evoke episodes of fibrillation/tachycardia. Chronic tachypacing-induced cardiomyopathy has been applied on its own to study the occurrence of spontaneous ventricular arrhythmias and was proven rather effective: after 5 weeks, six out of 25 dogs had died of sudden cardiac death, with polymorphic ventricular tachycardia as the recorded culprit on chronic Holter recordings (Pak et al., 1997). In combination with mitral regurgitation, chronic tachypacing has also shown effective as a model for atrial fibrillation. After 6 weeks, all dogs showed spontaneous persistent atrial fibrillation (Mitchell et al., 1997).

Of course, there are also models for electrical heart disease without induction of heart failure. Dogs with drug-induced long QT syndrome (LQTS) and kidney disease achieved by renal infarction are highly inducible for ventricular arrhythmias by pacing protocols or sympathetic challenge (Gallacher et al., 2007; Levine et al., 1985; Tang et al., 2017). LQTS and kidney disease are also well-known clinical conditions that predispose the occurrence of ventricular arrhythmias. Atrial fibrillation can be induced by pacing protocols quite effectively when combined with, for example, parasympathetic modulation, creation of an atrial lesion, or induction of systemic/pericardial inflammation. However, the fact that atrial fibrillation can be provoked after electrical stimulation does not necessarily mean that that model is a good reflection of the clinical situation. Atrial fibrillation has been well described in the context of pericarditis and systemic inflammation, but it is questionable whether a surgically created scar in the atrium or stimulation of the cervical vagal trunk is a good clinical reflection (Frame et al., 1986; Pagé et al., 1986; Wang et al., 1992; Yu et al., 2010).

Some naturally occurring diseases in dogs are also suitable for studying heart failure and electrical heart disease. Firstly, hereditary arrhythmogenic cardiomyopathy in boxers closely resembles the human syndrome. Nineteen out of 23 dogs showed spontaneous ventricular arrhythmias and post-mortem investigation demonstrated typical fibrofatty replacement of the myocardium (Basso et al., 2004). Hereditary dilating cardiomyopathy in Portuguese water dogs also showed typical post-mortem signs of dilating cardiomyopathy (Werner et al., 2008). Both breeds can be applied to study (early) interventions that can alter the course of the disease.

Finally, there is a residual category of less common causes of heart failure. Intraperitoneal infusion of *Trypanosoma cruzi* can be performed to induce Chagas cardiomyopathy or, secondly, intra-coronary infusion of the cardiotoxic **Adriamycin® (doxorubicin)** can be performed to bring about drug-induced cardiomyopathy. However uncommon, these heart failure models are reliable and have a phenotype that is close to its human counterpart (Carvalho et al., 2019; Magovern et al., 1992).

One dog model that has contributed enormously to our understanding of cardiac pro- and anti-arrhythmogenicity in response to drug application is the chronic atrioventricular block (CAVB) dog, which will be the further focus of this review.

4 | A SHORT HISTORY OF THE CHRONIC ATRIOVENTRICULAR BLOCK (CAVB) DOG

Shortly after the discovery of auricular–ventricular conduction pathway and its muscular nature by Wilhelm His Jr. and Sunao Tawara in the late 90s of the 19th and beginning of the 20th century (His, 1893; Tawara, 1906), attempts were made to create heart block, or currently named as atrial-ventricular block, in dogs in Europe (e.g. Hering, 1905) and the United States (e.g. Erlanger, 1906) (Figure 1). The first chronic AV block (CAVB) dogs were produced by Erlanger and Blackman (1910) in the years 1906 and 1907, as an experimental model of Stokes–Adams disease. In humans, this disease is characterized by periods of low pulse rates and syncope, due to AV conduction disturbance. They succeeded to create CAVB in four animals, which

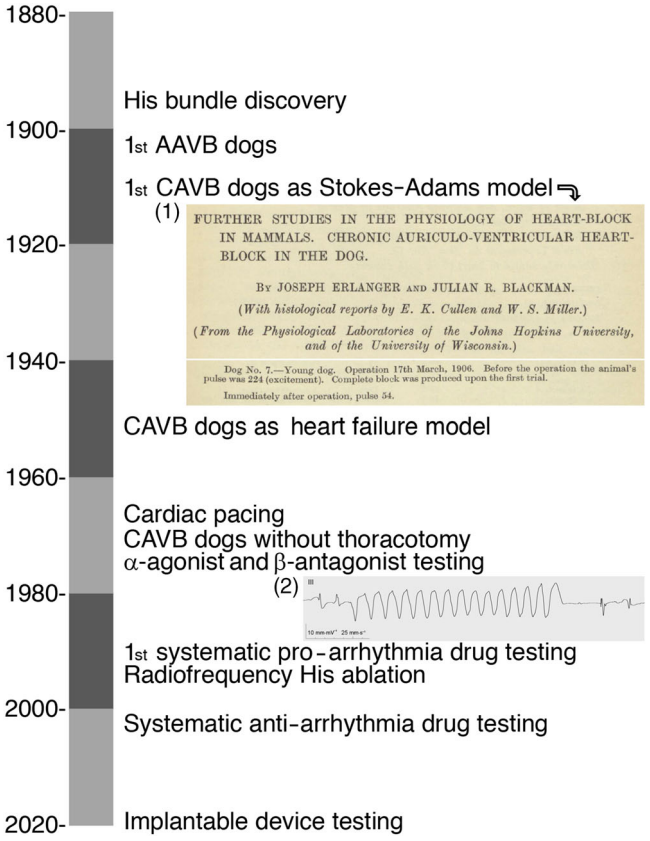

FIGURE 1 Timeline of chronic atrioventricular block (CAVB) dog development and usage in pharmacological research. Inserts: (1) Title page of the landmark Erlanger and Blackman (1910) paper and description of the creation of AV block in dog number 7 on 17th of March 1906. This dog experienced 'syncopal attacks' and was found dead in the morning of 15th of April 1906, after 4 weeks of permanent AV block. (2) ECG recording (Einthoven III) of a self-terminating drug-induced Torsade de Pointes arrhythmia

lasted up to 343 days in one dog. Of note are their observations that the animals behaved normally after they had recovered from the invasive procedure, however the AV block was irreversible and the hearts became hypertrophic. Furthermore, they observed that 'animals with complete heart block exhibit many interesting heart irregularities …' and spells of tachycardia. Two animals experienced syncopal attacks 'probably not determined by central processes' and sudden death, suggestive of lethal ventricular arrhythmia in retrospect. Remarkably, several of the basic observations made from the CAVB dog by Erlanger and Black are now, 100 years later, still not completely understood on a mechanistic level.

During the following years, methods for inducing AV block became less invasive and more selective (Boucher & Duchene-Marullaz, 1985). Whereas earliest procedures required thoracotomy combined with compression, sectioning and/or crushing of the His bundle area, later methods used (catheter) assisted injection of formalin or alcohol. Currently, radiofrequency ablation is the standard method for creating complete and irreversible AV block.

Since the Erlanger and Blackman paper, we could not obtain any reports on canine CAVB animals until the study of Starzl and Gaertner from 1955. They produced CAVB in dogs to establish a heart failure model. The majority of animals developed 'clinical, laboratory, and pathological evidence of heart failure spontaneously or after … exercise' (Starzl & Gaertner, 1955). In the 1960s, the CAVB model was used for testing compounds that either inhibited or stimulated noradrenergic (sympathetic) drive of the heart. Haemodynamic and, to some extent, electrophysiological effects were studied. In the 1980s, the conscious CAVB dog was used in toxicity and anti-arrhythmic research for which ventricular tachycardia induced by digitalis or **ouabain** infusion combined with ventricular pacing was taken as readout (Gorgels et al., 1987; Vos et al., 1989). From the early 1990s onwards, the model was used for systematic studies on drug-induced pro-arrhythmia with Torsade de Pointes (TdP) as an endpoint (Weissenburger et al., 1991) (Figure 1). Torsade de Pointes is a polymorphic ventricular arrhythmia characterized on the ECG by a twisting QRS morphology around the isoelectric line (Vos et al., 1998). The latest developments that make use of the CAVB model are found in implantable device development and testing (e.g. Smoczyńska et al., 2020).

5 | CHARACTERISTICS OF THE CAVB DOG AND ITS USE IN PHARMACOLOGY RESEARCH

Blocking the electrical conduction between the atria and ventricles results in an acute and permanent decrease in ventricular beating rate to an idioventricular rhythm (Figure 2). Due to the immediate drop in cardiac output as a result of bradycardia, a series of ventricular adjustments are initiated. The Frank–Starling principle and accompanying neurohumoral activation leads to a rapid increase in contractile force and hence stroke volume (Vos et al., 1998). At a slower timescale, structural remodelling associates with a gradual development of biventricular hypertrophy that contributes to the need for an increased stroke volume (Verduyn et al., 2001). Electrical remodelling, i.e. adjusting functional expression of ion channels and transporters that include a decrease in I_{Na}, I_{Kr} and I_{Ks} and an increase in Na^+/H^+ exchanger activity, results in lengthening of the action potential duration, increased intracellular Na^+ levels, (Na^+–Ca^{2+} exchanger [NCX] dependent) calcium (over)load and thus to increased contraction force and duration (Sipido et al., 2000; Van Borren et al., 2013; Verdonck et al., 2003; Volders et al., 1999). Whereas cardiac output is successfully restored to up to 90%, the remodelling processes come at a price: an increased vulnerability for drug-induced Torsade de Pointes arrhythmias (Vos et al., 1998). The decrease in expression of channels that conduct repolarizing currents challenges the repolarization reserve. The concept of repolarization reserve postulates that impairment of one type of ion channel does not excessively change repolarization duration and thus is not (easily) recognizable using standard diagnostic tools, like ECG parameters. Only multiple hits, for example, application of an ion channel inhibiting drug on top of hypokalaemia, dietary components, genetic predisposition or certain disease states, would excessively prolong repolaration and induce cardiac arrhythmia (for a comprehensive review on this topic, see Varró & Baczkó, 2011). The decrease in repolarization reserve can be quantified using short-term variability of repolarization (STV). STV is a measure of beat-to-beat changes in action potential duration, that is, temporal dispersion of ventricular repolarization, over a certain number of beats. Arrhythmia severity was found to associate with the steepness of STV

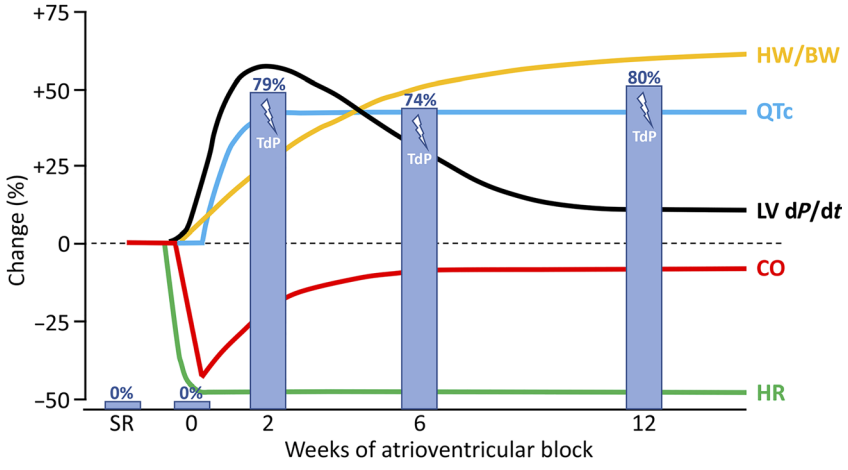

FIGURE 2 Depiction of cardiac changes in the chronic atrioventricular block (CAVB) dog in time following AV block, compared with the sinus rhythm (SR) animal. See text for further explanation. Bar graphs represent Torsade de Pointes(TdP) inducibility. CO, cardiac output; HR, heart rhythm; HW/BW, heart weight/body weight ratio; LV d*P*/d*t*, left ventricular pressure

increase just before Torsade de Pointes occurrence (Smoczyńska et al., 2019). In addition to displaying a clear increase prior to Torsade de Pointes occurrence, STV can identify CAVB dogs at risk for development of arrhythmias. The dogs that have proven inducible for Torsade de Pointes have a higher baseline STV than their non-inducible counterparts. This makes STV highly sensitive and specific in assessing the pro-arrhythmic risk of drugs and identification of individuals at risk. Compared with QT, which is still most commonly used for identifying drugs at risk for inducing ventricular arrhythmias, STV is superior (Varkevisser et al., 2012). Moreover, several clinical studies demonstrate the usefulness of STV as a predictor of human arrhythmias (Hinterseer et al., 2009). In addition to down-regulation of potassium channels, more factors contribute to the abnormal repolarization in the CAVB dog. The aforementioned alterations in calcium handling lead to increased intracellular calcium concentrations. This further enhances the prolongation of the action potential duration, as also brought about by the down-regulation of **cardiac potassium channels**. If the intracellular calcium surpasses a threshold, it can trigger early after depolarizations, which can evoke Torsade de Pointes under the right circumstances. Moreover, the bradycardic conditions during CAVB further enhance variability in repolarization. Experimental variables, for example, anaesthesia or low potassium plasma levels, challenge the delicate electrical balance of the remodelled ventricles even more (Dunnink et al., 2010; Thomsen, Volders, et al., 2006; Weissenburger et al., 1991). Furthermore, the ectopic foci that initiate each ventricular beat alter the normal ventricular activation pattern. This exaggerates electrical remodelling and arrhythmia susceptibility (Winckels et al., 2007).

Another parameter to quantify the increased sensitivity for Torsade de Pointes in the CAVB dog model is spatial dispersion of repolarization (SDR). SDR appraises local differences in repolarization duration within the ventricle. It develops, among other factors, in the setting of biventricular hypertrophy and heterogeneous electrical remodelling. By detailed ventricular mapping, it was found that **dofetilide**-induced Torsade de Pointes initiates in a region with highest SDR (Dunnink et al., 2017). Moreover, SDR is one of the factors that is believed to promote the formation of re-entry circuits. This is highly relevant in the formation of severe arrhythmias. Short lasting Torsade de Pointes are maintained by focal activity, whereas non-self-terminating Torsade de Pointes are maintained by re-entry mechanisms (Vandersickel et al., 2017). Van Weperen et al. (2019) underlined the importance of SDR in arrhythmogenesis as they demonstrated that the anti-arrhythmic efficacy of four highly effective anti-arrhythmic drugs was best reflected by SDR. STV, however still superior to the QT interval, only partially reflects the susceptibility to Torsade de Pointes. They concluded that SDR and STV are separately involved in arrhythmogenesis. The exact interplay however between spatial and temporal dispersion of repolarization in the formation of arrhythmias remains to be elucidated (Van Weperen et al., 2019).

Finally, the autonomic nervous system is a known factor that contributes to cardiac arrhythmias. Remodelling of the diseased heart is associated with nerve sprouting, which leads to a more heterogeneous cardiac innervation. As the autonomic nervous system affects the electrophysiology of the heart directly to adapt its frequency, contractility, conduction velocity and relaxation to altering conditions, it is no surprise that an altered innervation has an influence on arrhythmogenic susceptibility (Cao et al., 2000). More specifically, sympathetic activity shows a causal relation with the emergence of arrhythmias. This is attributed to the ability of the sympathetic overexcitation to trigger early afterdepolarizations. It is however unknown whether the autonomic nervous system is a factor that modulates STV and SDR, or is a separate factor in the complex equation that leads to the formation of arrhythmias (Shen & Zipes, 2014).

All in all, the CAVB dog is mainly considered as a model of triggered activity, although re-entry-based arrhythmias are present too.

In the remodelled dog heart, drugs that either block or activate, for example, certain ion channels, transporters or release channels can easily and rapidly induce Torsade de Pointes arrhythmias, which forms the basis of the successful application of the CAVB dog in safety pharmacology (Tables 2 and 3). On the other hand, anti-arrhythmic compounds that can target the same sets of proteins improve electrical stability or interfere with Torsade de Pointes progression and can prevent or suppress drug-induced arrhythmia, making the CAVB dog a versatile and valuable model in anti-arrhythmic drug development (Table 4).

6 | SAFETY PHARMACOLOGY

Due to its high sensitivity for drug-induced Torsade de Pointes arrhythmias, the CAVB dog model has been used in many safety pharmacology studies, either in the awake (Table 2) or anaesthetized state (Table 3). In conscious dog studies, the electrophysiological parameters, including Torsade de Pointes, were mostly determined using a Holter recording and analysis system. Drugs were either applied p.o. or i.v. at the start of a 24-h recording period. Unfortunately, according to the current *British Journal of Pharmacology* guidelines, studies in the awake conditions are often underpowered by inclusion of less than five animals. In anaesthetized dog studies, electrophysiological parameters are mostly determined by 6-lead surface ECG and catheter-mediated endocardial monophasic action potential recording. In these studies, drugs are mostly applied i.v. Many of the studies indicate a dose–response relationship for cardiotoxic drugs, whereas safe drugs are free of Torsade de Pointes even in 10-fold increase of clinical dosing. In some studies, using non-anaesthetized animals, drug sensitivity is further enhanced by diuretics-mediated hypokalaemia. Only three drugs, **lidocaine**, **moxifloxacin** and **D-sotalol**, have been tested in both the awake and anaesthetized conditions in a safety set-up. Lidocaine and D-sotalol had similar outcomes. However, the moxifloxacin studies gave conflicting outcomes, that is, absence of Torsade de Pointes in the anaesthetized state versus Torsade de Pointes induction in the awake state, with reported plasma levels even higher in the former condition. However, the means of application, i.v. versus p.o., respectively, differed. Finally, many outcomes from the

TABLE 2 Cardiac safety testing of drugs and compounds in the awake canine CAVB model

Drug[a]	Dose[b,c]	Peak plasma	TdP[b,c]	Reference
Amiodarone	3 mg·kg^{-1} p.o.	<50 ng·ml^{-1}	0/4	Yoshida et al., 2002
	30 mg·kg^{-1} p.o.	1082 ± 188 ng·ml^{-1}	0/4	Yoshida et al., 2002
	200 mg·day^{-1} p.o. for 7 days + 100 mg·day^{-1} p.o. for 21 days	Not reported	0/4	Takahara et al., 2008
Amlodipine	0.25 mg·kg^{-1} p.o. 4 weeks	24.22 ± 3.99 ng·ml^{-1}	0/8	Takahara et al., 2009
Apomorphine	1 mg·kg^{-1} 10 min^{-1} i.v.	3.5 ± 0.8 μg·ml^{-1}	0/4	Watanabe et al., 2015
Astemizol	3 mg·kg^{-1} p.o.	Not reported	0/4	Izumi-Nakaseko et al., 2016
	30 mg·kg^{-1} p.o.	Not reported	1/4	Izumi-Nakaseko et al., 2016
Azithromycin	30 mg·kg^{-1} 10 min^{-1} i.v.	68.5 ± 4.9 μg·ml^{-1}	0/4	Ohara et al., 2015
Bepridil	3 mg·kg^{-1} p.o.	Not reported	0/4	Takahara et al., 2008
	30 mg·kg^{-1} p.o.	Not reported	3/4	Takahara et al., 2008
Candesartan	1.2 mg·kg^{-1} p.o. 4 weeks	Not reported	0/7	Takahara et al., 2009
Cilnidipine	0.5 mg·kg^{-1} p.o. 4 weeks	9.16 ± 1.44 ng·ml^{-1}	0/7	Takahara et al., 2009
Cisapride	1 mg·kg^{-1} p.o.	Not reported	1/6	Sugiyama, Ishida, et al., 2002
	10 mg·kg^{-1} p.o.	Not reported	6/6	Sugiyama, Ishida, et al., 2002
	10–20 mg·kg^{-1} p.o.	Not reported	4/8	Wijers et al., 2018
E-4031	0.03 mg·kg^{-1} 10 min^{-1} i.v.	16.5 ng·ml^{-1}	0/4	Goto et al., 2018
	0.1 mg·kg^{-1} 10 min^{-1} i.v.	60.5 ng·ml^{-1}	1/4	Goto et al., 2018
	0.3 mg·kg^{-1} 10 min^{-1} i.v.	182.5 ng·ml^{-1}	4/4	Goto et al., 2018
Donepezil	0.1 mg·kg^{-1} 10 min^{-1} i.v.	Not reported	0/4	Hagiwara-Nagasawa et al., 2021
	1 mg·kg^{-1} 10 min^{-1} i.v.	Not reported	0/4	Hagiwara-Nagasawa et al., 2021
Famotidine	1 mg·kg^{-1} 5 min^{-1} i.v.	Not reported	0/4	Sugiyama et al., 2003
	10 mg·kg^{-1} 5 min^{-1} i.v.	Not reported	0/4	Sugiyama et al., 2003
Flecainide[d]	1.5 mg·kg^{-1} 10 min^{-1} + 0.9 mg·kg^{-1} 60 min^{-1} i.v.	0.7 ± 0.1 mg·L^{-1}	0/6	Weissenburger et al., 1991
	3 mg·kg^{-1} 10 min^{-1} + 1.8 mg·kg^{-1} 60 min^{-1} i.v.	Not reported	0/6	Weissenburger et al., 1991
Gatifloxacin	10 mg·kg^{-1} p.o.	4.1 ± 0.3 μg·ml^{-1}	0/4	Chiba et al., 2004
	100 mg·kg^{-1} p.o.	11.3 ± 1.6 μg·ml^{-1}	2/4	Chiba et al., 2004
Haloperidol	3 mg·kg^{-1} p.o.	Not reported	0/4	Izumi-Nakaseko et al., 2017
	30 mg·kg^{-1} p.o.	Not reported	4/4	Izumi-Nakaseko et al., 2017
Lapatinib	3 mg·kg^{-1} 10 min^{-1} i.v.	2.358 ± 0.424 μg·ml^{-1}	0/4	Ando et al., 2020
Levofloxacin	6 mg·kg^{-1} p.o.	1.81 ± 0.45 μg·ml^{-1}	0/4	Chiba et al., 2000
	60 mg·kg^{-1} p.o.	17.74 ± 02.57 μg·ml^{-1}	0/4	Chiba et al., 2000
Lidocaine[d]	3 mg·kg^{-1} 10 min^{-1} + 3 mg·kg^{-1} 60 min^{-1} i.v.	3.1 ± 0.6 mg·L^{-1}	0/6	Weissenburger et al., 1991
Mexiletine[d]	4.5 mg·kg^{-1} 10 min^{-1} + 1.5 mg·kg^{-1} 60 min^{-1} i.v.	Not reported	0/8	Chézalviel-Guilbert et al., 1995
Moxifloxacin	10 mg·kg^{-1} p.o.	2.1 ± 0.3 mg·ml^{-1}	0/4	Chiba et al., 2004
	100 mg·kg^{-1} p.o.	12.6 ± 1.0 μg·ml^{-1}	3/4	Chiba et al., 2004
Nifekalant	3 mg·kg^{-1} p.o.	4.66 ± 0.21 μg·ml^{-1}	0/5	Satoh et al., 2004
Oseltamivir	3 mg·kg^{-1} 10 min^{-1} i.v.	Not reported	0/4	Nakamura et al., 2016
(Tamiflu®)	10 mg·kg^{-1} 10 min^{-1} i.v.	Not reported	0/4	Nakamura et al., 2016
	30 mg·kg^{-1} 10 min^{-1} i.v.	Not reported	0/4	Nakamura et al., 2016
Propranolol	0.5 mg·kg^{-1} 70 min^{-1} i.v.	0.21 ± 0.03 mg·L^{-1}	0/6	Weissenburger et al., 1991
Quinidine[d]	10 mg·kg^{-1} 10 min^{-1} + 1.8 mg·kg^{-1} 60 min^{-1} i.v.	4.2 ± 0.8 mg·L^{-1}	0/6	Weissenburger et al., 1991
Risperidone	3 mg·kg^{-1} 10 min^{-1} i.v.	Not reported	0/4	Nunoi et al., 2020
Sematilide	3 mg·kg^{-1} p.o.	Not reported	0/4	Yoshida et al., 2002
	30 mg·kg^{-1} p.o.	Not reported	3/4	Yoshida et al., 2002
D-Sotalol	3 mg·kg^{-1} p.o.	Not reported	1/4	Goto, Hagiwara-Nagasawa, Chiba, et al., 2019

(Continues)

TABLE 2 (Continued)

Drug[a]	Dose[b,c]	Peak plasma	TdP[b,c]	Reference
D,L-Sotalol	3 mg·kg^{-1} p.o.	Not reported	3/4	Goto, Hagiwara-Nagasawa, Chiba, et al., 2019
	10 mg·kg^{-1} p.o.	Not reported	3/4	Goto, Hagiwara-Nagasawa, Kambayashi, et al., 2019
	2.25 mg·kg^{-1} 10 min^{-1} + 0.75 mg·kg^{-1} 60 min^{-1} i.v.	Not reported	1/5	Weissenburger et al., 1991
	4.5 mg·kg^{-1} 10 min^{-1} + 1.5 mg·kg^{-1} 60 min^{-1} i.v.	4.5 ± 0.2 mg·L^{-1}	5/6	Weissenburger et al., 1991
D-Sotalol[d]	4.5 mg·kg^{-1} 10 min^{-1} + 1.5 mg·kg^{-1} 60 min^{-1}	Not reported	6/7	Chézalviel-Guilbert et al., 1998
D-Sotalol +	4.5 mg·kg^{-1} 10 min^{-1} + 1.5 mg·kg^{-1} 60 min^{-1} i.v.	Not reported	1–3/7	Chézalviel-Guilbert et al., 1998
Quinidine[d]	10 mg·kg^{-1} 10 min^{-1} + 1.8 mg·kg^{-1} 60 min^{-1} i.v.	Not reported		
Sitafloxacin	10 mg·kg^{-1} p.o.	1.7 ± 0.4 μg·ml^{-1}	0/4	Chiba et al., 2004
	100 mg·kg^{-1} p.o.	9.8 ± 1.7 μg·ml^{-1}	0/4	Chiba et al., 2004
Sparfloxacin	6 mg·kg^{-1} p.o.	1.56 ± 0.15 μg·ml^{-1}	0/4	Chiba et al., 2000
	60 mg·kg^{-1} p.o.	3.89 ± 1.39 μg·ml^{-1}	4/4	Chiba et al., 2000
Sulpiride	6 mg·kg^{-1} p.o.	Not reported	0/4	Sugiyama, Satoh, et al., 2002
	60 mg·kg^{-1} p.o.	Not reported	1/4	Sugiyama, Satoh, et al., 2002
	120 mg·kg^{-1} p.o.	Not reported	2/4	Sugiyama, Satoh, et al., 2002
Terfenadine	3 mg·kg^{-1} p.o.	Not reported	1/6	Takahara et al., 2006
	30 mg·kg^{-1} p.o	Not reported	5/6	Takahara et al., 2006

[a]In case a drug was tested in multiple studies, publications were selected that provided the most comprehensive dataset.
[b,c]Only studies with a defined drug dosing regimen and TdP as explicit endpoint were selected.
[d]Hypokalaemic conditions.

studies in anaesthetized animals were obtained in a prevention set-up of anti-arrhythmic drug testing (e.g. flunarizine, K201, **ranolazine** and **SEA0400**), prior to dofetilide infusion.

In general, there is a very good concordance between the absence or occurrence of drug-induced Torsade de Pointes in the CAVB dog, either awake or anaesthetized and clinical findings (e.g. lidocaine, e Silva et al., 2018; **cisapride**, Hennessy et al., 2008; sotalol, Haverkamp et al., 1997; and dofetilide, Torp-Pedersen et al., 1999).

7 | ANTI-ARRHYTHMICS TESTED IN THE CAVB DOG MODEL

In addition to safety pharmacology testing, the CAVB dog model has proven useful in testing the efficacy of anti-arrhythmic drugs. These experiments can be performed as either suppression or prevention experiment. In suppression experiments, dogs are challenged with a drug (usually a potassium channel blocker) to evoke Torsade de Pointes. After the occurrence of a predefined arrhythmic endpoint (e.g. three Torsade de Pointes within 10 minutes or one Torsade de Pointes overall, depending on the experiment), the tested anti-arrhythmic substance is infused to test if the substance can abrogate the rhythm disturbances. For prevention experiments, an initial experiment is necessary to test if a dog indeed shows Torsade de Pointes after the infusion of a challenging drug. If so, this dog is considered 'inducible' and will proceed to the actual prevention experiment. During this experiment, the tested anti-arrhythmic substance is infused shortly before, or co-infused with, the challenging drug to test if the occurrence of Torsade de Pointes can be prevented.

All drugs that have been specifically developed to diminish ventricular arrhythmias and that have been tested in the CAVB dog model are presented in Table 4. As can be appreciated, arrangement of the drugs in Table 4 is based on which ion channel is targeted to exert its mode of action. Moreover, the majority of the listed experiments are performed in a suppression set-up (S). Drugs targeting calcium channels directly (e.g. **verapamil**, Oros et al., 2010) are highly effective in suppressing Torsade de Pointes, whereas the efficacy of downstream targeting of calcium handling is lower (Bourgonje et al., 2013). Overall, the anti-arrhythmic effect of sodium channel inhibition is good, but anti-arrhythmic efficacy varies between these drugs. The potassium channel activators, like I_{Kr} (Qile et al., 2019) and I_{KATP} targeting drugs (Thomsen, Volders, et al., 2006; Watanabe et al., 2015), lastly, have also proven to be effective in suppressing and preventing Torsade de Pointes.

Interestingly, the QT interval only shortened significantly after anti-arrhythmic therapy in a minority of the anti-arrhythmic drug interventions. This underlines the shortcoming of the QT interval as a measure for risk of arrhythmic events, as it does not reflect the reduction in the incidence of Torsade de Pointes in these experiments

TABLE 3 Cardiac safety testing of drugs and compounds in the anaesthetized canine CAVB model

Drug[a]	Dose[b,c]	Peak plasma	TdP[b,c]	Reference
Almokalant	0.12 mg·kg^{-1} 10 min^{-1} i.v.	Not reported	9/14	Verduyn et al., 1997
Amiodarone	40 mg·kg^{-1} for 28 days p.o.	3.5 ± 0.6 mg·L^{-1}	0/7	Van Opstal, Schoenmakers, et al., 2001
AVE0118	0.5 mg·kg^{-1} 5 min^{-1} i.v.	0.55 ± 0.1 μg·ml^{-1}	0/5	Oros et al., 2006
	3 mg·kg^{-1} 60 min^{-1} i.v.	1.9 ± 0.5 μg·ml^{-1}	0/5	Oros et al., 2006
	10 mg·kg^{-1} 60 min^{-1} i.v.	6.1 ± 1.2 μg·ml^{-1}	0/5	Oros et al., 2006
AZD1305	1.08 mg·kg^{-1} 30 min^{-1} i.v.	1.77 ± 0.29 μM	4/11	Johnson et al., 2012
Azimilide	10 mg·kg^{-1} 5 min^{-1} i.v.	Not reported	5/9	Van Opstal, Leunissen, et al., 2001
Azithromycin	2 mg·kg^{-1} 5 min^{-1} i.v.	5.4 ± 1.3 μg·ml^{-1}	0/5	Thomsen, Beekman, et al., 2006
	8 mg·kg^{-1} 5 min^{-1} i.v.	20.8 ± 4.9 μg·ml^{-1}	0/5	Thomsen, Beekman, et al., 2006
DHE	0.33 mg·kg^{-1} 5 min^{-1} i.v.	1.2 μM	2/4	Baburin et al., 2018
	0.5 mg·kg^{-1} 5 min^{-1} i.v.	2.3 μM	0/4	Baburin et al., 2018
Dofetilide	25 μg·kg^{-1} 5 min^{-1} i.v.	80 nM	10/13	Thomsen et al., 2003
Dronedarone	2 × 20 mg·kg^{-1} for 28 days p.o.	1.3 ± 0.3 mg·L^{-1}	4/8	Van Opstal, Schoenmakers, et al., 2001
Flunarizine	2 mg·kg^{-1} 2 min^{-1} i.v.	Not reported	0/8	Oros et al., 2010
Ibutilide	25 μg·kg^{-1} 5 min^{-1} i.v.	Not reported	6/10	Boulaksil et al., 2011
Istaroxime	180 μg·kg^{-1} 60 min^{-1} i.v.	Not reported	0/7	Bossu, Kostense, et al., 2018
K201	0.1 mg·kg^{-1} 2 min^{-1} + 0.01 mg·kg^{-1} 30 min^{-1} i.v.	450 ± 100 nM	0/7	Stams et al., 2011
	0.3 mg·kg^{-1} 2 min^{-1} + 0.03 mg·kg^{-1} 30 min^{-1} i.v.	1080 ± 350 nM	3/7	Stams et al., 2011
Lidocaine	3 mg·kg^{-1} 2 min^{-1} i.v.	Not reported	0/7	Antoons et al., 2010
LUF7244	2.5 mg·kg^{-1} 15 min^{-1} i.v.	2.34 ± 1.57 μM	0/7	Qile et al., 2019
Moxifloxacin	2 mg·kg^{-1} 5 min^{-1} i.v.	4.6 ± 2.5 μg·ml^{-1}	0/6	Thomsen, Beekman, et al., 2006
	8 mg·kg^{-1} 5 min^{-1} i.v.	22.0 ± 6.8 μg·ml^{-1}	0/6	Thomsen, Volders, et al., 2006
NS-7	3 mg·kg^{-1} 5 min^{-1} i.v.	1.6 ± 1.9 μg·ml^{-1}	3/6	Detre et al., 2005
PA-6	2.5 mg·kg^{-1} 10 min^{-1} i.v.	5.33 ± 0.63 μM	0/9	Ji et al., 2017
Ranolazine	4 mg·kg^{-1} 0.5 min^{-1} + 5.6 mg·kg^{-1} 25 min^{-1} i.v.	22.8 ± 2.3 μM	0/6	Antoons et al., 2010
SEA0400	0.4 mg·kg^{-1} 5 min^{-1} i.v.	5 ± 1 μM	0/3	Bourgonje et al., 2013
	0.8 mg·kg^{-1} 5 min^{-1} i.v.	11 ± 2 μM	0/4	Bourgonje et al., 2013
Sertindole	0.2 mg·kg^{-1} 5 min^{-1} i.v.	Not reported	0/9	Thomsen, Volders, et al., 2006
	1.0 mg·kg^{-1} 5 min^{-1} i.v.	1.3 ± 0.2 μM	3/5	Thomsen et al., 2003
D-Sotalol	2 mg·kg^{-1} 5 min^{-1} i.v.	Not reported	2/8	Thomsen et al., 2004
	4 mg·kg^{-1} 5 min^{-1} i.v.	Not reported	6/8	Thomsen et al., 2004
Verapamil	0.3 mg·kg^{-1} 5 min^{-1} i.v.	Not reported	0/3	Bourgonje et al., 2013
	0.4 mg·kg^{-1} 3 min^{-1} i.v.	Not reported	0/7	Oros et al., 2010
Vernakalant	2 mg·kg^{-1} 10 min^{-1} i.v.	Not reported	0/8	Varkevisser et al., 2013

[a]In case a drug was tested in multiple studies, publications were selected that provided the most comprehensive dataset.
[b,c]Only studies with a defined drug dosing regimen and TdP (Torsade de Pointes)as explicit endpoint were selected.

(Bossu et al., 2017). STV performs better as an indicator for arrhythmic risk in these experiments, as it decreased significantly after anti-arrhythmic treatment in the majority of the interventions. However, as STV does not decrease significantly in all experiments, it is not perfect. Apparently, and not unexpected, additional factors play a part in the determination of the arrhythmic threat.

As mentioned above, Table 4 summarizes the efficacy of drugs that have been designed to treat ventricular arrhythmias. Therefore, some drugs that have also been tested in a prevention or suppression set-up in the CAVB, but were not primarily designed to target ventricular arrhythmias, have been omitted. An example is istaroxime, a positive inotropic drug. As current inotropic agents are notorious for inducing ventricular arrhythmias, this study should be primarily considered as safety pharmacology testing rather than anti-arrhythmic testing. It is thus not surprising that istaroxime displayed only a mild preventive effect on the occurrence of Torsade de Pointes (6/6 vs. 4/6) (Bossu, Kostense, et al., 2018). The same goes for the drugs K201 and AVE0118, which were designed to target atrial fibrillation.

TABLE 4 Overview of tested anti-arrhythmic drugs in the CAVB dog model

Compound	Ion channel target	Dose (i.v.)	TdP occurrence		QTc (ms)		STV (ms)		Reference
			Challenge	Suppression/ prevention	Challenge	Suppression/ prevention	Challenge	Suppression/ prevention	
Flunarizine (S)	Calcium channel inhibitor	2 $mg \cdot kg^{-1}$ 2 min^{-1}	10/10	0/10	553 ± 40	425 ± 38*	4.5 ± 1.5	1.5 ± 0.6*	Oros et al., 2010
Verapamil (S)	Calcium channel inhibitor	0.4 $mg \cdot kg^{-1}$ 3 min^{-1}	7/7	0/7	566 ± 87	516 ± 90	3.2 ± 1.1	1.5 ± 0.7*	Oros et al., 2010
W-7 (S)	Calmodulin inhibitor	50 $\mu mol \cdot kg^{-1}$ 5 min^{-1}	6/6	2/6	523 ± 69	489 ± 88	3.1 ± 1.1	2.7 ± 1.6	Bourgonje et al., 2012
SEA0400 (S)	Sodium–calcium exchanger inhibitor	0.8 $mg \cdot kg^{-1}$ 5 min^{-1}	4/4	0/4	549 ± 95	702 ± 45	12.0 ± 6.4	7.3 ± 3.2	Bourgonje et al., 2013
GS-458967 (S)	Late sodium current inhibitor	0.1 $mg \cdot kg^{-1}$ 5 min^{-1}	7/7	0/7	609 ± 44	551 ± 77*	4.2 ± 2.5	2.7 ± 0.9	Bossu, Houtman, et al., 2018
Lidocaine (S)	Sodium channel inhibitor	3 $mg \cdot kg^{-1}$ 2 min^{-1}	6/6	2/6	489 ± 41	503 ± 72	3.6 ± 0.8	2.3 ± 0.9*	Antoons et al., 2010
Ranolazine (S)	Sodium channel inhibitor	4 $mg \cdot kg^{-1}$ 5 min^{-1} + 5.6 $mg \cdot kg^{-1}$ 25 min^{-1}	5/5	2/5	523 ± 69	489 ± 88	4.5 ± 0.8	3.2 ± 0.5*	Antoons et al., 2010
Mexiletine (S)	Sodium channel inhibitor	4.5 $mg \cdot kg^{-1}$ + 1.5 $mg \cdot kg^{-1} \cdot h^{-1}$ for 2.5 h	6/8	2/8	nr	nr	nr	nr	Chézalviel-Guilbert et al., 1995
LUF7244 (P)	Delayed rectifier potassium channel activator	2.5 $mg \cdot kg^{-1}$ 15 min^{-1}	7/7	2/7	498 ± 44	544 ± 48	3.3 ± 2.0	7.6 ± 6.7	Qile et al., 2019
Levcromakalim (S)	ATP-sensitive potassium channel opener	3 $\mu g \cdot kg^{-1}$ 3 min^{-1}	7/7	2/7	460 ± 69	481 ± 120	4.9 ± 2.1	2.6 ± 0.9*	Thomsen, Volders, et al., 2006
Nicorandil (S)	ATP-sensitive potassium channel opener	1–1.5 $mg \cdot kg^{-1}$ 5 min^{-1}	4/10	0/10	nr	nr	nr	nr	Watanabe et al., 2011

Note: Summarizes the different anti-arrhythmic drugs tested in the CAVB dog model. The selected references serve as an illustrative example and do not represent a comprehensive list of all the studies that have tested the drug in the CAVB dog model.

Abbreviations: CAVB, chronic atrioventricular block; nr, not reported; (P), prevention experiment; (S), suppression experiment; STV, short-term variability of repolarization; TdP, Torsade de Pointes arrhythmia.

* denotes significant difference between challenge and prevention/suppression.

As their modes of action might predispose to Torsade de Pointes, these studies should also be primarily considered as safety pharmacology testing (Oros et al., 2006; Stams et al., 2011).

8 | STRENGTHS, WEAKNESSES AND PERSPECTIVE OF THE CAVB DOG MODEL

Due to its concordance to human cardiac physiology, the dog is one of the species recommended by the FDA for *in vivo* cardiotoxicity evaluation (ICH S7B). When comparing a single drug in multiple systems, it was found that the safe I_{Kr} blocker moxifloxacin, at therapeutical concentrations, did not induce Torsade de Pointes arrhythmias in the anaesthetized CAVB dog and **methoxamine**-sensitized rabbit, whereas it did induce early after depolarization (considered as an arrhythmogenic endpoint) in isolated adult cardiomyocytes from rabbit and the CAVB dog (Nalos et al., 2012). Furthermore, the CAVB dog is a sensitive model for non-I_{Kr} blocking drugs, like those that inhibit the inward rectifier channel (e.g. PA-6, Ji et al., 2017), sodium channel (e.g. lidocaine, Weissenburger et al., 1991), L-type calcium channel (e.g. verapamil, Oros et al., 2010) or even multiple channel blockers (e.g. flunarizine, Oros et al., 2010). Another benefit of the model is its applicability for serial testing due to its high reproducibility of, for example, dofetilide-induced Torsade de Pointes arrhythmias between subsequent experiments (Oros et al., 2008). This characteristic can be exploited in a prevention set-up, in which a test drug is infused in an 'inducible' CAVB dog, followed by dofetilide. Furthermore, multiple compounds can be tested subsequently for arrhythmia inducibility and other electrophysiological parameters within the same animal, allowing robust drug comparisons. Thirdly, in line with inducibility is the remarkable finding that approximately 20%–25% of the CAVB dogs display no Torsade de Pointes following dofetilide infusion, whereas all animals undergo similar experimental treatments (Oros et al., 2008). This reflects, to some extent, the human observation that drug-induced arrhythmia is only apparent in predisposed people, according to the repolarization reserve concept. The CAVB dogs mostly have a mixed genetic background and, in that aspect, mirror the human population. Given the available dog genome sequence, one can envision genotype–phenotype comparisons between inducible and non-inducible animals for a defined set of genes involved in cardiac electrophysiology, which may provide genetic information that determines susceptibility for drug-induced arrhythmia in these animals and potentially in humans. Fourthly, *in vivo* and *in vitro* testing can be performed using the same animal. Whereas arrhythmogenicity scoring can display different outcomes, as indicated above, effects on repolarization duration and many other parameters can be compared. Furthermore, *in vivo* observations can be further analysed using isolated cells from the same animal. For example, ranolazine was tested as preventing dofetilide-induced arrhythmia *in vivo* and subsequently was demonstrated to inhibit late sodium current in isolated cardiomyocytes, despite a partial down-regulation of late sodium current in the CAVB dog (Antoons et al., 2010). Moreover, the predictive value of cardiac safety testing in the CAVB dog model is, after so many years of experience, well characterized (Gralinski, 2003). Finally, a practical benefit of the CAVB model is its size. This allows use of standard clinical tools like catheters, pacemakers, implantable cardioverter-defibrillators (ICDs) and echocardiography probes. It will also provide sufficiently large plasma amounts for multiple tests and isolated cardiomyocytes for subsequent analysis.

Obviously, the CAVB model also comes with several limitations. First of all, it is an expensive system. A single purpose bred experimental dog, including housing and experimentation, will cost approximately €4000. In an academic setting, these costs per animal obviously will not allow large *n*-numbers, despite the ability for serial testing. Not only is the use of dogs in research costly, but it is also labour intensive requiring specific expertise, the remodelling process is time consuming and there are many demands to comply with appropriate housing for dogs. Many studies with dogs are being performed in collaboration with the pharmaceutical industry, and therefore, study variables and outcomes could be confidential until scientific publication of the results, which hampers rapid academic exchange of preliminary results. Secondly, societal acceptance for the use of dogs in research is challenged, in the past but even more today and requires continuous stringent justification within ethical committees (obviously) and to the public. This challenged societal acceptance can lead to a diminished openness on research in dogs to the public. The ethical and cost constraints have most likely also contributed to the reluctance for developing genetically engineered dog models, whereas these have been developed in other larger animal models such as rabbit, pigs and goats (Baczkó et al., 2020; Li et al., 2016; Polejaeva et al., 2016). This could limit future developments of dog models that mimic the clinical situation more accurately, such as atherosclerosis-associated ischaemia and rhythm disturbances.

Due to the millennia of coexistence and breeding programmes, the dog is considered as man's best friend. However, its physiology as indicated above is still different in several aspects compared with humans, which still gives rise to a translational gap. Moreover, Torsade de Pointes arrhythmias are a very specific type of polymorphic ventricular arrhythmias that are clinically only observed in a setting of prolonged repolarization and bradycardia (Al-Khatib et al., 2018). Torsade de Pointes is the classic type of arrhythmia that is associated with the congenital LQTS. Congenital LQTS is a cardiac repolarization disorder characterized by a prolonged QT interval, caused by a critical mutation in one of the cardiac ion channels, with a prevalence of about 0.5% (Schwartz et al., 2009). The concordance between the CAVB dog model and the human congenital LQTS is thus a diminished repolarization capacity that can give rise to Torsade de Pointes arrhythmias. However, the contractile and structural remodelling that occur in the CAVB dog are different from this patient population. Heart failure, whether compensated or not, is no typical trait of the congenital LQTS patients (Vos et al., 1998). A transgene LQTS dog would closer approximate these patients. The structural remodelling leading to ventricular hypertrophy in the CAVB dog does show some phenotypical overlap with hypertrophic cardiomyopathy (HCM). Hypertrophic cardiomyopathy is caused by a mutation in contractile sarcomeric proteins, which leads to cardiac hypertrophy with a wide

array of clinical manifestations. Patients with hypertrophic cardiomyopathy, however, do not typically show QT prolongation, bradycardia or AV dyssynchrony, nor do they develop Torsade de Pointes arrhythmias. In fact, ventricular tachycardia, ventricular fibrillation and atrial fibrillation are the most common arrhythmias in this patient population. Moreover, hypertrophic cardiomyopathy patients display a decrease in cardiac contractility and develop cardiac fibrosis, whereas the opposite holds true for CAVB dogs (Elliott & McKenna, 2004). Also in this case, a genetically engineered dog model would give a better approximation of this patient population. All in all, the canine CAVB model does reproduce some aspects of hereditary LQTS and hypertrophic cardiomyopathy, but the differences are too extensive to reproduce these syndromes completely. Clinically, ventricular tachycardia and ventricular fibrillation are observed far more often than Torsade de Pointes and therefore make up a larger contribution to sudden cardiac death than Torsade de Pointes. It is thus questionable to which extent obtained insights into the mechanisms underlying Torsade de Pointes are applicable to target sudden cardiac death in humans in general (Al-Khatib et al., 2018). Contributing to this uncertainty is that the evoked Torsade de Pointes in the CAVB dog model are majorly triggered activity based, as can be explained by the alterations in calcium handling and sympathetic innervation as explained above. As the formation of re-entry circuits is also a well-established factor in the formation and perpetuation of ventricular arrhythmias in humans, this could further enhance the translational gap that the CAVB dog model poses in finding targets for diminishing life-threatening arrhythmias (El-Sherif et al., 1981).

The use of the CAVB dog in preclinical research has dropped significantly over the past years. Taking the costs, required expertise, ethical constraints, lack of transgenic opportunities and translational obstacles into account, its use will probably drop even more. It will become more and more difficult for the academia to cover the costs that using the CAVB dog model entails. In this regard, collectives such as the Health and Environmental Sciences Institute (HESI) are interesting, which is currently supporting the CiPA initiative. Their aim is to centrally unite academic, industry and government and regulatory parties to resolve health challenges. This would encompass combining the expertise of the academy with the funds of the industry and, by means of centralization, improving the generalizability of the dog model.

In this regard, it is also important to place the CAVB dog in the perspective of other available animal models in preclinical cardiac research. Firstly, mouse models have the major advantage that they are very suitable for genetic modification. Given the short gestation and large litters, they can produce many of transgene animals in a short time. However apt to develop transgene models and gain insight in certain mechanisms, the mouse is not very suitable further on in the translation process in the arrhythmogenic research field. The mouse action potential is very different from the human, due to a different expression of cardiac ion channels and altered relative contributions of calcium and potassium currents to the action potential. Moreover, mice have a resting heart rate of 600–800 bpm and a different cardiovascular anatomy (Clauss et al., 2019). The electrophysiological properties of rabbits however are rather similar to the human situation, especially in the ventricles. Almost all major human ion currents are also present in rabbit ventricular myocytes, with some differences in relative contribution and therefore a fairly comparable ventricular action potential waveform. The atrial electrophysiology however differs significantly from humans. This renders rabbits very suitable for studies on ventricular repolarization. Several transgenic LQTS rabbit models have been developed, in addition to a CAVB rabbit model. The CAVB rabbit displays a high incidence of spontaneous Torsade de Pointes (approximately 70%) and the transgenic LQTS rabbits vary, depending on the type of mutation, from exerting spontaneous VT to a reduced repolarization capacity, which needs an additional hit to promote Torsade de Pointes (Baczkó et al., 2020; Clauss et al., 2019; Tsuji et al., 2002). The costs of rabbits are lower and appropriate housing is easier to arrange than for dogs. Additionally, transgenic models are an option, even though not many are available yet. However, no data on reproducibility of arrhythmia induction in the same animal are available, and therefore, serial testing is not possible, contrary to the CAVB dog. This reduces the financial benefit of the initially cheaper rabbits and limits certain study set-ups. Moreover, the rabbit CAVB model encompasses a failing heart and requires immediate pacing following acute AV block, contrary to the compensated canine CAVB model and the differences in size and anatomy further limit the rabbit's translation into clinical practice. Although built almost 20 years ago (e.g. Tsuji et al., 2002), the number of publications using the CAVB rabbit model in preclinical drug research is scarce when compared with the dog CAVB model, for unknown reasons. Finally, the predictive value of preclinical research in rabbits has not been characterized, contrary to the CAVB dog model. Rabbit models could however be a future option for cardiac safety and repolarization-related research but need to be further developed. The use of pigs in preclinical research is also more socially accepted than dogs and there is an option to make transgene models as well. The pig does have a less similar anatomy of the conduction system and make-up of ion currents to humans, compared with dogs. The coronary anatomy and atrial electrical properties on the other hand are more similar to humans (Clauss et al., 2019). They could be considered to validate data from rabbit models, to bridge the translational gap that the rabbits' size, anatomy and atrial electrophysiology pose. Finally, as mentioned before, human cardiomyocytes derived from induced pluripotent stem cells carrying rare diseases could be applied to evaluate cellular and molecular mechanisms. As they lack the complex integration of cardiac electrical activity, pump function, haemodynamic feedback, neural regulation, electrolyte homeostasis, display of SDR and their collective response to pharmacological treatment, the known targets for these cardiomyocytes should be further investigated from here on.

Underlining the complexity of advancing in the anti-arrhythmic research field, only a few papers have been published on new anti-arrhythmic targets in the last decade. For example, **SK2 ($K_{Ca}2.2$)** channels and connexin43-based gap junctions were added to the potential anti-arrhythmic repertoire (Hamilton et al., 2020; Lucero et al., 2020). Obviously, knowledge is progressing but at its best at a modest pace.

9 | CONCLUSIONS

Understanding of the mechanistic processes underlying cardiac arrhythmia in the CAVB dog is steadily progressing, but slowly. The complex and multifactorial cardiac compensatory remodelling process upon CAVB provides multiple targets on which drugs may act to induce cardiotoxicity. The CAVB model is placed in the latter stages of the drug development process and its integrated outcome, that is, cardiac arrhythmia, can be mirrored against more reductionistic endpoints from dedicated *in silico* and *in vitro* models. Outcomes of cardiac safety testing in the CAVB dog demonstrate good concordance with clinical observations on adverse drug effects. The CAVB dog is amenable for evaluating anti-arrhythmic compounds with diverse molecular targets. The combination of determining *in vivo* and *in vitro* parameters from the same model strengthens mechanistic insights and potentially translating power. Use of the model is challenged by its costs, societal acceptance and the limited occurrence of Torsade de Pointes (the specific type of arrhythmia that is evoked by the CAVB dog model) in clinical practice. Therefore, the mechanistic insights into the occurrence and perpetuation of arrhythmias that have arisen from the CAVB dog might be limited outside cardiac safety and anti-arrhythmic testing. Moreover, the lack of (opportunity to develop) transgene models will limit future developments that can improve the translational gap. We therefore expect that the use of the CAVB model will be under increasing pressure. Nevertheless, the CAVB model deserves its important role in drug-related cardiac arrhythmia research. Compared with other available *in vitro* and *in vivo* systems, the CAVB dog model is best characterized in relation to clinical observations. The other available models, however promising, should be further developed and validated with clinical data before they can replace the CAVB dog model completely. In the meantime, centralized collaborations between academic, industrial and government parties can help cover the increasing costs, combine expertise and improve generalizability of drug target validation and safety screening using the CAVB dog model.

9.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the IUPHAR/BPS Guide to PHARMACOLOGY http://www.guidetopharmacology.org and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander et al., 2019).

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AUTHOR CONTRIBUTIONS

V.L. and M.v.d.H. designed the review, collected and analysed the literature and wrote the draft manuscript. M.V. critically reviewed the draft version and contributed to the final version. All authors read and approved the final version of the manuscript.

CONFLICT OF INTERESTS

M.V. is supported by collaborations with Medtronic and Amgen. V.L. and M.v.d.H. declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable—no new data are generated.

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REFERENCES

Alexander, S. P. H., Mathie, A., Peters, J. A., Veale, E. L., Striessnig, J., Kelly, E., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Sharman, J. L., Southan, C., Davies, J. A., Aldrich, R. W., Becirovic, E., Biel, M., Catterall, W. A., Conner, A. C., Davies, P., … Zhu, M. (2019). The Concise Guide to PHARMACOLOGY 2019/20: Ion channels. *British Journal of Pharmacology*, *176*(S1). https://doi.org/10.1111/bph.14749

Al-Khatib, S. M., Stevenson, W. G., Ackerman, M. J., Bryant, W. J., Callans, D. J., Curtis, A. B., Deal, B. J., Dickfeld, T., Field, M. E., Fonarow, G. C., Gillis, A. M., Granger, C. B., Hammill, S. C., Hlatky, M. A., Joglar, J. A., Kay, G. N., Matlock, D. D., Myerburg, R. J., & Page, R. L. (2018). 2017 AHA/ACC/HRS guideline for management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: A report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Rhythm Society. *Circulation*, *138*, e272–e391. https://doi.org/10.1161/CIR.0000000000000549

Ando, K., Wada, T., & Cao, X. (2020). Precise safety pharmacology studies of lapatinib for onco-cardiology assessed using in vivo canine models. *Scientific Reports*, *10*, 738. https://doi.org/10.1038/s41598-020-57601-x

Antoons, G., Oros, A., Beekman, J. D., Engelen, M. A., Houtman, M. J., Belardinelli, L., Stengl, M., & Vos, M. A. (2010). Late Na^+ current inhibition by ranolazine reduces torsades de pointes in the chronic atrioventricular block dog model. *Journal of the American College of Cardiology*, *55*, 801–809. https://doi.org/10.1016/j.jacc.2009.10.033

Arita, T., Sorescu, G. P., Schuler, B. T., Schmarkey, L. S., Merlino, J. D., Vinten-Johansen, J., Leon, A. R., Martin, R. P., & Sorescu, D. (2007). Speckle-tracking strain echocardiography for detecting cardiac dyssynchrony in a canine model of dyssynchrony and heart failure. *American Journal of Physiology. Heart and Circulatory Physiology*, *293*, H735–H742. https://doi.org/10.1152/ajpheart.00168.2007

Armstrong, P. W., Stopps, T. P., Ford, S. E., & de Bold, A. J. (1986). Rapid ventricular pacing in the dog: Pathophysiologic studies of heart failure. *Circulation*, *74*, 1075–1084. https://doi.org/10.1161/01.CIR.74.5.1075

Baburin, I., Varkevisser, R., Schramm, A., Saxena, P., Beyl, S., Szkokan, P., Linder, T., Stary-Weinzinger, A., van der Heyden, M. A. G., Houtman, M., Takanari, H., Jonsson, M., Beekman, J. H. D., Hamburger, M., Vos, M. A., & Hering, S. (2018). Dehydroevodiamine and hortiamine, alkaloids from the traditional Chinese herbal drug *Evodia rutaecarpa*, are I_{Kr} blockers with proarrhythmic effects in vitro

and in vivo. *Pharmacological Research*, *131*, 150–163. https://doi.org/10.1016/j.phrs.2018.02.024

Baczkó, I., Hornyik, T., Brunner, M., Koren, G., & Odening, K. E. (2020). Transgenic rabbit models in proarrhythmia research. *Frontiers in Pharmacology*, *11*, 853. https://doi.org/10.3389/fphar.2020.00853

Basso, C., Fox, P. R., Meurs, K. M., Towbin, J. A., Spier, A. W., Calabrese, F., Maron, B. J., & Thiene, G. (2004). Arrhythmogenic right ventricular cardiomyopathy causing sudden cardiac death in boxer dogs: A new animal model of human disease. *Circulation*, *109*, 1180–1185. https://doi.org/10.1161/01.CIR.0000118494.07530.65

Bossu, A., Houtman, M. J. C., Meijborg, V. M. F., Varkevisser, R., Beekman, H. D. M., Dunnink, A., de Bakker, J. M. T., Mollova, N., Rajamani, S., Belardinelli, L., van der Heyden, M. A. G., & Vos, M. A. (2018). Selective late sodium current inhibitor GS-458967 suppresses Torsades de Pointes by mostly affecting perpetuation but not initiation of the arrhythmia. *British Journal of Pharmacology*, *175*, 2470–2482. https://doi.org/10.1111/bph.14217

Bossu, A., Kostense, A., Beekman, H. D. M., Houtman, M. J. C., van der Heyden, M. A. G., & Vos, M. A. (2018). Istaroxime, a positive inotropic agent devoid of proarrhythmic properties in sensitive chronic atrioventricular block dogs. *Pharmacological Research*, *133*, 132–140. https://doi.org/10.1016/j.phrs.2018.05.001

Bossu, A., Varkevisser, R., Beekman, H. D. M., Houtman, M. J. C., van der Heyden, M. A. G., & Vos, M. A. (2017). Short-term variability of repolarization is superior to other repolarization parameters in the evaluation of diverse antiarrhythmic interventions in the chronic atrioventricular block dog. *Journal of Cardiovascular Pharmacology*, *69*, 398–407. https://doi.org/10.1097/FJC.0000000000000488

Boucher, M., & Duchene-Marullaz, P. (1985). Methods for producing experimental complete atrioventricular block in dogs. *Journal of Pharmacological Methods*, *13*, 95–107. https://doi.org/10.1016/0160-5402(85)90053-1

Boulaksil, M., Jungschleger, J. G., Antoons, G., Houtman, M. J., de Boer, T. P., Wilders, R., Beekman, J. D., Maessen, J. G., van der Hulst, F. F., van der Heyden, M. A. G., van Veen, T. A. B., van Rijen, H. V. M., de Bakker, J. M. T., & Vos, M. A. (2011). Drug-induced torsade de pointes arrhythmias in the chronic AV block dog are perpetuated by focal activity. *Circulation. Arrhythmia and Electrophysiology*, *4*, 566–576. https://doi.org/10.1161/CIRCEP.110.958991

Bourgonje, V. J., Schoenmakers, M., Beekman, J. D., van der Nagel, R., Houtman, M. J., Miedema, L. F., Antoons, G., Sipido, K., de Windt, L. J., van Veen, T. A. B., & Vos, M. A. (2012). Relevance of calmodulin/CaMKII activation for arrhythmogenesis in the AV block dog. *Heart Rhythm*, *9*, 1875–1883. https://doi.org/10.1016/j.hrthm.2012.07.023

Bourgonje, V. J., Vos, M. A., Ozdemir, S., Doisne, N., Acsai, K., Varro, A., Sztojkov-Ivanov, A., Zupko, I., Rauch, E., Kattner, L., Bito, V., Houtman, M., van der Nagel, R., Beekman, J. D., van Veen, T. A. B., Sipido, K. R., & Antoons, G. (2013). Combined Na^+/Ca^{2+} exchanger and L-type calcium channel block as a potential strategy to suppress arrhythmias and maintain ventricular function. *Circulation. Arrhythmia and Electrophysiology*, *6*, 371–379. https://doi.org/10.1161/CIRCEP.113.000322

Brams, W. A., & Katz, L. N. (1931). The nature of experimental flutter and fibrillation of the heart. *American Heart Journal*, *7*, 249–261. https://doi.org/10.1016/S0002-8703(31)90415-8

Camm, A. J. (2017). Hopes and disappointments with antiarrhythmic drugs. *International Journal of Cardiology*, *237*, 71–74. https://doi.org/10.1016/j.ijcard.2017.03.056

Cao, J. M., Chen, L. S., KenKnight, B. H., Ohara, T., Lee, M. H., Tsai, J., Lai, W. W., Karagueuzian, H. S., Wolf, P. L., Fishbein, M. C., & Chen, P. S. (2000). Nerve sprouting and sudden cardiac death. *Circulation Research*, *86*, 816–821. https://doi.org/10.1161/01.RES.0000133678.22968.e3

Carvalho, E. B., Ramos, I. P. R., Nascimento, A. F. S., Brasil, G. V., Mello, D. B., Oti, M., Sammeth, M., Bahia, M. T., Campos de Carvalho, A. C., & Carvalho, A. B. (2019). Echocardiographic measurements in a preclinical model of chronic Chagasic cardiomyopathy in dogs: Validation and reproducibility. *Frontiers in Cellular and Infection Microbiology*, *9*, 332. https://doi.org/10.3389/fcimb.2019.00332

Chézalviel-Guilbert, F., Davy, J. M., Poirier, J. M., & Weissenburger, J. (1995). Mexiletine antagonizes effects of sotalol on QT interval duration and its proarrhythmic effects in a canine model of torsade de pointes. *Journal of the American College of Cardiology*, *26*, 787–792. https://doi.org/10.1016/0735-1097(95)00234-U

Chézalviel-Guilbert, F., Deplanne, V., Davy, J. M., Poirier, J. M., Xia, Y. Z., Cheymol, G., & Weissenburger, J. (1998). Combination of sotalol and quinidine in a canine model of torsades de pointes: No increase in the QT-related proarrhythmic action of sotalol. *Journal of Cardiovascular Electrophysiology*, *9*, 498–507. https://doi.org/10.1111/j.1540-8167.1998.tb01842.x

Chiba, K., Sugiyama, A., Hagiwara, T., Takahashi, S., Takasuna, K., & Hashimoto, K. (2004). In vivo experimental approach for the risk assessment of fluoroquinolone antibacterial agents-induced long QT syndrome. *European Journal of Pharmacology*, *486*, 189–200. https://doi.org/10.1016/j.ejphar.2003.12.014

Chiba, K., Sugiyama, A., Satoh, Y., Shiina, H., & Hashimoto, K. (2000). Proarrhythmic effects of fluoroquinolone antibacterial agents: In vivo effects as physiologic substrate for torsades. *Toxicology and Applied Pharmacology*, *169*, 8–16. https://doi.org/10.1006/taap.2000.9041

Clauss, S., Bleyer, C., Schüttler, D., Tomsits, P., Renner, S., Klymiuk, N., Wakili, R., Massberg, S., Wolf, E., & Kääb, S. (2019). Animal models of arrhythmia: Classic electrophysiology to genetically modified large animals. *Nature Reviews. Cardiology*, *16*, 457–475. https://doi.org/10.1038/s41569-019-0179-0

Cox, J. L., Canavan, T. E., Schuessler, R. B., Cain, M. E., Lindsay, B. D., Stone, C., Smith, P. K., Corr, P. B., & Boineau, J. P. (1991). The surgical treatment of atrial fibrillation. II. Intraoperative electrophysiologic mapping and description of the electrophysiologic basis of atrial flutter and atrial fibrillation. *The Journal of Thoracic and Cardiovascular Surgery*, *101*, 406–426.

Damiano, B. P., van der Linde, H. J., Van Deuren, B., Somers, Y., Lubomirski, M., Teisman, A., & Gallacher, D. J. (2015). Characterization of an anesthetized dog model of transient cardiac ischemia and rapid pacing: A pilot study for preclinical assessment of the potential for proarrhythmic risk of novel drug candidates. *Journal of Pharmacological and Toxicological Methods*, *72*, 72–84. https://doi.org/10.1016/j.vascn.2014.10.006

Detre, E., Thomsen, M. B., Beekman, J. D., Petersen, K. U., & Vos, M. A. (2005). Decreasing the infusion rate reduces the proarrhythmic risk of NS-7: Confirming the relevance of short-term variability of repolarisation in predicting drug-induced torsades de pointes. *British Journal of Pharmacology*, *145*, 397–404. https://doi.org/10.1038/sj.bjp.0706203

Dunnink, A., Sharif, S., Oosterhoff, P., Winckels, S., Montagne, D., Beekman, J., van der Nagel, R., van der Heyden, M. A. G., & Vos, M. A. (2010). Anesthesia and arrhythmogenesis in the chronic atrioventricular block dog model. *Journal of Cardiovascular Pharmacology*, *55*, 601–608. https://doi.org/10.1097/FJC.0b013e3181da7768

Dunnink, A., Stams, T. R. G., Bossu, A., Meijborg, V. M. F., Beekman, J. D. M., Wijers, S. C., de Bakker, J. M. T., & Vos, M. A. (2017). Torsade de pointes arrhythmias arise at the site of maximal heterogeneity of repolarization in the chronic complete atrioventricular block dog. *Europace*, *19*, 858–865. https://doi.org/10.1093/europace/euw087

Durrer, D., & Van der Tweel, L. H. (1957). Excitation of the left ventricular wall of the dog and goat. *Annals of the New York Academy of Sciences*, *65*, 779–803. https://doi.org/10.1111/j.1749-6632.1957.tb36683.x

e Silva, L. O. J., Scherber, K., Cabrera, D., Motov, S., Erwin, P. J., West, C. P., Murad, M. H., & Bellolio, M. F. (2018). Safety and efficacy of intravenous lidocaine for pain management in the emergency department: A systematic review. *Annals of Emergency Medicine*, *72*(2), 135–144.e3. https://doi.org/10.1016/j.annemergmed.2017.12.014

Elliott, P., & McKenna, W. J. (2004). Hypertrophic cardiomyopathy. *Lancet*, *363*, 1881–1891. https://doi.org/10.1016/S0140-6736(04)16358-7

El-Sherif, N., Scherlag, B. J., Lazzara, R., & Hope, R. R. (1977). Re-entrant ventricular arrhythmias in the late myocardial infarction period. 1. Conduction characteristics in the infarction zone. *Circulation*, *55*, 686–702. https://doi.org/10.1161/01.CIR.55.5.686

El-Sherif, N., Smith, R. A., & Evans, K. (1981). Canine ventricular arrhythmias in the late myocardial infarction period. Epicardial mapping of reentrant circuits. *Circulation Research*, *49*, 255–265. https://doi.org/10.1161/01.RES.49.1.255

Erlanger, J. (1906). On the physiology of heart-block in mammals, with especial reference to the causation of Stokes-Adams disease. *The Journal of Experimental Medicine*, *8*, 8–58. https://doi.org/10.1084/jem.8.1.8

Erlanger, J., & Blackman, J. R. (1910). Further studies in the physiology of heart-block in mammals. Chronic auriculo-ventricular heart-block in the dog. *Heart*, *1*, 177–229.

Eyster, J. A. E., & Swarthout, E. C. (1920). Experimental determination of the influence of the abnormal cardiac rhythms of on the mechanical efficiency of the heart. *Archives of Internal Medicine (Chicago, Ill.)*, *25*, 317–324. https://doi.org/10.1001/archinte.1920.00090320088007

Feld, G. K., & Shahandeh-Rad, F. (1992). Activation patterns in experimental canine atrial flutter produced by right atrial crush injury. *JACC*, *20*, 441–451. https://doi.org/10.1016/0735-1097(92)90115-4

Feola, M., Haiderer, O., & Kennedy, J. H. (1971). Experimental graded "pump failure" of the left ventricule. *The Journal of Surgical Research*, *11*, 325–341. https://doi.org/10.1016/0022-4804(71)90110-7

Frame, L. H., Page, R. L., & Hoffman, B. F. (1986). Atrial reentry around an anatomic barrier with a partially refractory excitable gap. A canine model of atrial flutter. *Circulation Research*, *58*, 495–511. https://doi.org/10.1161/01.res.58.4.495

Fukuda, K., Kanazawa, H., Aizawa, Y., Ardell, J. L., & Shivkumar, K. (2015). Cardiac innervation and sudden cardiac death. *Circulation Research*, *116*, 2005–2019. https://doi.org/10.1161/CIRCRESAHA.116.304679

Gaasch, W. H., Zile, M. R., Hoshino, P. K., Apstein, C. S., & Blaustein, A. S. (1989). Stress-shortening relations and myocardial blood flow in compensated and failing canine hearts with pressure-overload hypertrophy. *Circulation*, *79*, 872–883. https://doi.org/10.1161/01.cir.79.4.872

Gallacher, D. J., Van de Water, A., Van der Linde, H., Hermans, A. H., Lu, H. R., Towart, R., & Volders, P. G. (2007). In vivo mechanisms precipitating torsades de pointes in a canine model of drug-induced long-QT1 syndrome. *Cardiovascular Research*, *76*, 247–256. https://doi.org/10.1016/j.cardiores.2007.06.019

Gintant, G., Sager, P. T., & Stockbridge, N. (2016). Evolution of strategies to improve preclinical cardiac safety testing. *Nature Reviews. Drug Discovery*, *15*, 457–471. https://doi.org/10.1038/nrd.2015.34

Gorgels, A. P., De Wit, B., Beekman, H. D., Dassen, W. R., & Wellens, H. J. (1987). Triggered activity induced by pacing during digitalis intoxication: Observations during programmed electrical stimulation in the conscious dog with chronic complete atrioventricular block. *Pacing and Clinical Electrophysiology*, *10*, 1309–1321. https://doi.org/10.1111/j.1540-8159.1987.tb04967.x

Goto, A., Hagiwara-Nagasawa, M., Chiba, K., Kambayashi, R., Nunoi, Y., Izumi-Nakaseko, H., Matsumoto, A., Kanda, Y., & Sugiyama, A. (2019). Pharmacological β-adrenoceptor blockade can augment torsadogenic action of I_{Kr} inhibitor: Comparison of proarrhythmic effects of *d*-sotalol and *dl*-sotalol in the chronic atrioventricular block dogs. *Journal of Pharmacological Sciences*, *141*, 86–89. https://doi.org/10.1016/j.jphs.2019.09.009

Goto, A., Hagiwara-Nagasawa, M., Kambayashi, R., Chiba, K., Izumi-Nakaseko, H., Naito, A. T., Kanda, Y., & Sugiyama, A. (2019). Measurement of J–T_{peak}c along with QT-interval prolongation may increase the assay sensitivity and specificity for predicting the onset of drug-induced torsade de pointes: Experimental evidences based on proarrhythmia model animals. *Cardiovascular Toxicology*, *19*, 357–364. https://doi.org/10.1007/s12012-019-09506-z

Goto, A., Izumi-Nakaseko, H., Hagiwara-Nagasawa, M., Chiba, K., Ando, K., Naito, A. T., & Sugiyama, A. (2018). Analysis of torsadogenic and pharmacokinetic profile of E-4031 in dogs bridging the gap of information between in vitro proarrhythmia assay and clinical observation in human subjects. *Journal of Pharmacological Sciences*, *137*, 237–240. https://doi.org/10.1016/j.jphs.2018.06.005

Gralinski, M. R. (2003). The dog's role in the preclinical assessment of QT interval prolongation. *Toxicologic Pathology*, *31*, 11–16. https://doi.org/10.1080/01926230390174887

Hagiwara-Nagasawa, M., Kambayashi, R., Goto, A., Nunoi, Y., Izumi-Nakaseko, H., Chiba, K., Wada, T., Takei, Y., Matsumoto, A., & Sugiyama, A. (2021). Analysis of electropharmacological and proarrhythmic effects of donepezil using the halothane-anesthetized intact dogs and the conscious chronic atrioventricular block ones. *Naunyn Schmiedebergs Archives Pharmacology*, *394*, 581–589. https://doi.org/10.1007/s00210-020-01997-w

Hamilton, S., Polina, I., Terentyeva, R., Bronk, P., Kim, T. Y., Roder, K., Clements, R. T., Koren, G., Choi, B. R., & Terentyev, D. (2020). PKA phosphorylation underlies functional recruitment of sarcolemmal SK2 channels in ventricular myocytes from hypertrophic hearts. *The Journal of Physiology*, *598*, 2847–2873. https://doi.org/10.1113/JP277618

Harris, A. S. (1950). Delayed development of ventricular ectopic rhythms following experimental coronary occlusion. *Circulation*, *1*, 1318–1328. https://doi.org/10.1161/01.CIR.1.6.1318

Haverkamp, W., Martinez-Rubio, A., Hief, C., Lammers, A., Mühlenkamp, S., Wichter, T., Breithardt, G., & Borggrefe, M. (1997). Efficacy and safety of *d*,*l*-sotalol in patients with ventricular tachycardia and in survivors of cardiac arrest. *Journal of the American College of Cardiology*, *30*, 487–495. https://doi.org/10.1016/s0735-1097(97)00190-3

He, K. L., Dickstein, M., Sabbah, H. N., Yi, G. H., Gu, A., Maurer, M., Wei, C. M., Wang, J., & Burkhoff, D. (2004). Mechanisms of heart failure with well preserved ejection fraction in dogs following limited coronary microembolization. *Cardiovascular Research*, *64*, 72–83. https://doi.org/10.1016/j.cardiores.2004.06.007

Hennessy, S., Leonard, C. E., Newcomb, C., Kimmel, S. E., & Bilker, W. B. (2008). Cisapride and ventricular arrhythmia. *British Journal of Clinical Pharmacology*, *66*, 375–385. https://doi.org/10.1111/j.1365-2125.2008.03249.x

Hering, H. E. (1905). Nachweis, dass das His'sche Uebergangsbündel Vorhof und Kammer des Säugethierherzens functionell verbindet. *Pflügers Archiv*, *108*, 267–280. https://doi.org/10.1007/BF01678340

Herr, M. D., McInerney, J. J., Copenhaver, G. L., & Morris, D. L. (1985). Coronary artery embolization in closed-chest canines using flexible radiopaque plugs. *Journal of Applied Physiology*, *64*, 2236–2239. https://doi.org/10.1152/jappl.1988.64.5.2236

Heusch, G., Guth, B. D., Seitelberger, R., & Ross, J. Jr. (1987). Attenuation of exercise-induced myocardial ischemia in dogs with recruitment of coronary vasodilator reserve by nifedipine. *Circulation*, *75*, 482–490. https://doi.org/10.1161/01.cir.75.2.482

Hinterseer, M., Beckmann, B. M., Thomsen, M. B., Pfeufer, A., Dalla Pozza, R., Loeff, M., Netz, H., Steinbeck, G., Vos, M. A., & Kääb, S. (2009). Relation of increased short-term variability of QT interval to congenital long-QT syndrome. *The American Journal of Cardiology*, *103*, 1244–1248. https://doi.org/10.1016/j.amjcard.2009.01.011

His, W. (1893). Die Tätigkeit des embryonalen herzens und deren bedeutung für die Lehre von der Hezebewegung beim Menschen. *Arb med Klinik Leipzig*, *1893*, 14–19.

ICH Guideline S7B. (May 2005). https://database.ich.org/sites/default/files/S7B_Guideline.pdf (assessed on 22 October 2020).

Izumi-Nakaseko, H., Nakamura, Y., Cao, X., Wada, T., Ando, K., & Sugiyama, A. (2016). Possibility as an anti-cancer drug of astemizole: Evaluation of arrhythmogenicity by the chronic atrioventricular block canine model. *Journal of Pharmacological Sciences*, *131*, 150–153. https://doi.org/10.1016/j.jphs.2016.04.024

Izumi-Nakaseko, H., Nakamura, Y., Cao, X., Wada, T., Ando, K., & Sugiyama, A. (2017). Assessment of safety margin of an antipsychotic drug haloperidol for torsade de pointes using the chronic atrioventricular block dogs. *Cardiovascular Toxicology*, *17*, 319–325. https://doi.org/10.1007/s12012-016-9388-5

Ji, Y., Varkevisser, R., Opacic, D., Bossu, A., Kuiper, M., Beekman, J. D. M., Yang, S., Khan, A. P., Dobrev, D., Voigt, N., Wang, M. Z., Verheule, S., Vos, M. A., & van der Heyden, M. A. G. (2017). The inward rectifier current inhibitor PA-6 terminates atrial fibrillation and does not cause ventricular arrhythmias in goat and dog models. *British Journal of Pharmacology*, *174*, 2576–2590. https://doi.org/10.1111/bph.13869

Johnson, D. M., de Jong, M. M., Crijns, H. J., Carlsson, L. G., & Volders, P. G. (2012). Reduced ventricular proarrhythmic potential of the novel combined ion-channel blocker AZD1305 versus dofetilide in dogs with remodeled hearts. *Circulation. Arrhythmia and Electrophysiology*, *5*, 201–209. https://doi.org/10.1161/CIRCEP.111.963025

Jost, N., Virág, L., Comtois, P., Ördög, B., Szuts, V., Seprényi, G., Bitay, M., Kohajda, Z., Koncz, I., Nagy, N., Szél, T., Magyar, J., Kovács, M., Puskás, L. G., Lengyel, C., Wettwer, E., Ravens, U., Nánási, P. P., Papp, J. G., … Nattel, S. (2013). Ionic mechanisms limiting cardiac repolarization reserve in humans compared to dogs. *The Journal of Physiology*, *591*, 4189–4206. https://doi.org/10.1113/jphysiol.2013.261198

Kleaveland, J. P., Kussmaul, W. G., Vinciguerra, T., Diters, R., & Carabello, B. A. (1988). Volume overload hypertrophy in a closed-chest model of mitral regurgitation. *The American Journal of Physiology*, *254*, H1034–H1041. https://doi.org/10.1152/ajpheart.1988.254.6.H1034

Levine, J. H., Spear, J. F., Guarnieri, T., Weisfeldt, M. L., de Langen, C. D., Becker, L. C., & Moore, E. N. (1985). Cesium chloride-induced long QT syndrome: Demonstration of afterdepolarizations and triggered activity in vivo. *Circulation*, *72*, 1092–1103. https://doi.org/10.1161/01.cir.72.5.1092

Li, D., Fareh, S., Leung, T. K., & Nattel, S. (1999). Promotion of atrial fibrillation by heart failure in dogs. Atrial remodeling of a different sort. *Circulation*, *100*, 87–95. https://doi.org/10.1161/01.CIR.100.1.87

Li, Y., Fuchimoto, D., Sudo, M., Haruta, H., Lin, Q. F., Takayama, T., Morita, S., Nochi, T., Suzuki, S., Sembon, S., Nakai, M., Kojima, M., Iwamoto, M., Hashimoto, M., Yoda, S., Kunimoto, S., Hiro, T., Matsumoto, T., Mitsumata, M., … Onishi, A. (2016). Development of human-like advanced coronary plaques in low-density lipoprotein receptor knockout pigs and justification for statin treatment before formation of atherosclerotic plaques. *Journal of the American Heart Association*, *5*, e002779. https://doi.org/10.1161/JAHA

Lucero, C. M., Andrade, D. C., Toledo, C., Díaz, H. S., Pereyra, K. V., Diaz-Jara, E., Schwarz, K. G., Marcus, N. J., Retamal, M. A., Quintanilla, R. A., & del Rio, R. (2020). Cardiac remodeling and arrhythmogenesis are ameliorated by administration of Cx43 mimetic peptide Gap27 in heart failure rats. *Scientific Reports*, *10*, 6878. https://doi.org/10.1038/s41598-020-63336-6

Ludwig, C. (1847). Beitrage zur Kenntniss des Einflusses der Respirationsbewegungen auf den Blutlauf im Aortensysteme. *Archives of Anatomy and Physiology*, *13*, 242–302.

Magovern, J. A., Christlieb, I. Y., Badylak, S. F., Lantz, G. C., & Kao, R. L. (1992). A model of left ventricular dysfunction caused by intracoronary adriamycin. *The Annals of Thoracic Surgery*, *53*, 861–863. https://doi.org/10.1016/0003-4975(92)91452-f

Mitchell, M. A., McRury, I. D., & Haines, D. E. (1997). Linear atrial ablations in a canine model of chronic atrial fibrillation. Morphological and electrophysiological observations. *Circulation*, *97*, 1176–1185. https://doi.org/10.1161/01.CIR.97.12.1176

Mizeres, N. J. (1955). The anatomy of the autonomic nervous system in the dog. *The American Journal of Anatomy*, *96*, 285–318. https://doi.org/10.1002/aja.1000960205

Moïse, N. S., Flanders, W. H., & Pariaut, R. (2020). Beat-to-beat patterning of sinus rhythm reveals non-linear rhythm in the dog compared to the human. *Frontiers in Physiology*, *10*, 1548. https://doi.org/10.3389/fphys.2019.01548

Morillo, C. A., Klein, G. J., Jones, D. L., & Guiraudon, C. M. (1995). Chronic rapid atrial pacing. Structural, functional, and electrophysiological characteristics of a new model of sustained atrial fibrillation. *Circulation*, *91*, 1588–1595. https://doi.org/10.1161/01.cir.91.5.1588

Munagala, V. K., Hart, C. Y., Burnett, J. C. Jr., Meyer, D. M., & Redfield, M. M. (2005). Ventricular structure and function in aged dogs with renal hypertension: A model of experimental diastolic heart failure. *Circulation*, *111*, 1128–1135. https://doi.org/10.1161/01.CIR.0000157183.21404.63

Nakamura, Y., Sasaki, R., Cao, X., Wada, T., Hamaguchi, S., Izumi-Nakaseko, H., Ando, K., Tanaka, H., Takahara, A., & Sugiyama, A. (2016). Intravenous anti-influenza drug oseltamivir will not induce torsade de pointes: Evidences from proarrhythmia model and action-potential assay. *Journal of Pharmacological Sciences*, *131*, 72–75. https://doi.org/10.1016/j.jphs.2016.04.018

Nalos, L., Varkevisser, R., Jonsson, M. K., Houtman, M. J., Beekman, J. D., van der Nagel, R., Thomsen, M. B., Duker, G., Sartipy, P., de Boer, T. P., Peschar, M., Rook, M. B., van Veen, T., van der Heyden, M., & Vos, M. A. (2012). Comparison of the I_{Kr} blockers moxifloxacin, dofetilide and E-4031 in five screening models of pro-arrhythmia reveals lack of specificity of isolated cardiomyocytes. *British Journal of Pharmacology*, *165*, 467–478. https://doi.org/10.1111/j.1476-5381.2011.01558.x

Nishida, K., Qi, X. Y., Wakili, R., Comtois, P., Chartier, D., Harada, M., Iwasaki, Y. K., Romeo, P., Maguy, A., Dobrev, D., Michael, G., Talajic, M., & Nattel, S. (2011). Mechanisms of atrial tachyarrhythmias associated with coronary artery occlusion in a chronic canine model. *Circulation*, *123*, 137–146. https://doi.org/10.1161/CIRCULATIONAHA.110.972778

Nunoi, Y., Chiba, K., Hagiwara-Nagasawa, M., Goto, A., Kambayashi, R., Izumi-Nakaseko, H., Takei, Y., Matsumoto, A., Watanabe, Y., & Sugiyama, A. (2020). Risperidone alone did not induce torsade de pointes: Experimental evidence from the chronic atrioventricular block model dogs. *Journal of Pharmacological Sciences*, *143*, 330–332. https://doi.org/10.1016/j.jphs.2020.05.008

Ohara, H., Nakamura, Y., Watanabe, Y., Cao, X., Yamazaki, Y., Izumi-Nakaseko, H., Ando, K., Yamazaki, H., Yamazaki, J., Ikeda, T., & Sugiyama, A. (2015). Azithromycin can prolong QT interval and suppress ventricular contraction, but will not induce torsade de pointes. *Cardiovascular Toxicology*, *15*, 232–240. https://doi.org/10.1007/s12012-014-9289-4

Ohara, K., Miyauchi, Y., Ohara, T., Fishbein, M. C., Zhou, S., Lee, M.-H., Mandel, W. J., Chen, P.-S., & Karagueuzian, H. S. (2002). Down-regulation of immunodetectable atrial connexin40 in a canine model of chronic left ventricular myocardial infarction: Implications to atrial fibrillation. *Journal of Cardiovascular Pharmacology and Therapeutics*, *7*, 89–94. https://doi.org/10.1177/107424840200700205

Oros, A., Beekman, J. D., & Vos, M. A. (2008). The canine model with chronic, complete atrio-ventricular block. *Pharmacology & Therapeutics*, *119*, 168–178. https://doi.org/10.1016/j.pharmthera.2008.03.006

Oros, A., Houtman, M. J., Neco, P., Gomez, A. M., Rajamani, S., Oosterhoff, P., Attevelt, N. J., Beekman, J. D., van der Heyden, M., ver Donck, L., Belardinelli, L., Richard, S., Antoons, G., Vos, M. A., & for the CONTICA investigators. (2010). Robust anti-arrhythmic efficacy of verapamil and flunarizine against dofetilide-induced TdP arrhythmias is based upon a shared and a different mode of action. *British Journal*

of Pharmacology, *161*, 162–175. https://doi.org/10.1111/j.1476-5381.2010.00883.x

Oros, A., Volders, P. G., Beekman, J. D., van der Nagel, T., & Vos, M. A. (2006). Atrial-specific drug AVE0118 is free of torsades de pointes in anesthetized dogs with chronic complete atrioventricular block. *Heart Rhythm*, *3*, 1339–1345. https://doi.org/10.1016/j.hrthm.2006.07.017

Pagé, P. L., Plumb, V. J., Okumura, K., & Waldo, A. L. (1986). A new animal model of atrial flutter. *Journal of the American College of Cardiology*, *8*, 872–879. https://doi.org/10.1016/s0735-1097(86)80429-6

Pak, P. H., Nuss, H. B., Tunin, R. S., Kääb, S., Tomaselli, G. F., Marban, E., & Kass, D. A. (1997). Repolarization abnormalities, arrhythmia and sudden death in canine tachycardia-induced cardiomyopathy. *Journal of the American College of Cardiology*, *30*, 576–584. https://doi.org/10.1016/s0735-1097(97)00193-9

Patel, S., Bhatt, L., Patel, R., Shah, C., Patel, V., Patel, J., Sundar, R., Bhatnagar, U., & Jain, M. (2017). Identification of appropriate QTc formula in beagle dogs for nonclinical safety assessment. *Regulatory Toxicology and Pharmacology*, *89*, 118–124. https://doi.org/10.1016/j.yrtph.2017.07.026

Po, S. S., Scherlag, B. J., Yamanashi, W. S., Edwards, J., Zhou, J., Wu, R., Geng, N., Lazzara, R., & Jackman, W. M. (2005). Experimental model for paroxysmal atrial fibrillation arising at the pulmonary vein-atrial junctions. *Heart Rhythm*, *3*, 201–820. https://doi.org/10.1016/j.hrthm.2005.11.008

Polejaeva, I. A., Ranjan, R., Davies, C. J., Regouski, M., Hall, J., Olsen, A. L., Meng, Q., Rutigliano, H. M., Dosdall, D. J., Angel, N. A., Sachse, F. B., Seidel, T., Thomas, A. J., Stott, R., Panter, K. E., Lee, P. M., Van Wettere, A. J., Stevens, J. R., Wang, Z., … White, K. L. (2016). Increased susceptibility to atrial fibrillation secondary to atrial fibrosis in transgenic goats expressing transforming growth factor-β1. *Journal of Cardiovascular Electrophysiology*, *27*, 1220–1229. https://doi.org/10.1111/jce.13049

Qile, M., Beekman, H. D. M., Sprenkeler, D. J., Houtman, M. J. C., van Ham, W. B., Stary-Weinzinger, A., Beyl, S., Hering, S., van den Berg, D.-J., de Lange, E. C. M., Heitman, L. H., IJzerman, A. P., Vos, M. A., & van der Heyden, M. A. G. (2019). LUF7244, an allosteric modulator/activator of K_v11.1 channels, counteracts dofetilide-induced torsades de pointes arrhythmia in the chronic atrioventricular block dog model. *British Journal of Pharmacology*, *176*, 3871–3885. https://doi.org/10.1111/bph.14798

Reimer, K. A., Jennings, R. B., Cobb, F. R., Murdock, R. H., Greenfield, J. C. Jr., Becker, L. C., Bulkley, B. H., Hutchins, G. M., Schwartz, R. P. Jr., & Bailey, K. R. (1985). Animal models for protecting ischemic myocardium: Results of the NHLBI Cooperative Study. Comparison of unconscious and conscious dog models. *Circulation Research*, *56*, 651–665. https://doi.org/10.1161/01.res.56.5.651

Sabbah, H. N., Stein, P. D., Kono, T., Gheorghiade, M., Levine, T. B., Jafri, S., Hawkins, E. T., & Goldstein, S. (1991). A canine model of chronic heart failure produced by multiple sequential coronary microembolizations. *The American Journal of Physiology*, *260*, H1379–H1384. https://doi.org/10.1152/ajpheart.1991.260.4.H1379

Saku, K., Kakino, T., Arimura, T., Sunagawa, G., Nishikawa, T., Sakamoto, T., Kishi, T., Tsutsui, H., & Sunagawa, K. (2018). Left ventricular mechanical unloading by total support of Impella in myocardial infarction reduces infarct size, preserves left ventricular function, and prevents subsequent heart failure in dogs. *Circulation. Heart Failure*, *11*, e004397. https://doi.org/10.1161/CIRCHEARTFAILURE.117.004397

Salazar, A. E. (1961). Experimental myocardial infarction. Induction of coronary thrombosis in the intact closed-chest dog. *Circulation Research*, *9*, 1351–1356. https://doi.org/10.1161/01.res.9.6.1351

Sasayama, S., Ross, J. Jr., Franklin, D., Bloor, C. M., Bishop, S., & Dilley, R. B. (1976). Adaptations of the left ventricle to chronic pressure overload. *Circulation Research*, *38*, 172–178. https://doi.org/10.1161/01.res.38.3.172

Satoh, Y., Sugiyama, A., Takahara, A., Chiba, K., & Hashimoto, K. (2004). Electropharmacological and proarrhythmic effects of a class III antiarrhythmic drug nifekalant hydrochloride assessed using the in vivo canine models. *Journal of Cardiovascular Pharmacology*, *43*, 715–723. https://doi.org/10.1097/00005344-200405000-00015

Schwartz, P. J., Stramba-Badiale, M., Crotti, L., Pedrazzini, M., Besana, A., Bosi, G., Gabbarini, F., Goulene, K., Insolia, R., Mannarino, S., Mosca, F., Nespoli, L., Rimini, A., Rosati, E., Salice, P., & Spazzolini, C. (2009). Prevalence of the congenital long-QT syndrome. *Circulation*, *120*, 1761–1767. https://doi.org/10.1161/CIRCULATIONAHA.109.863209

Shen, M. J., & Zipes, D. P. (2014). Role of the autonomic nervous system in modulating cardiac arrhythmias. *Circulation Research*, *114*, 1004–1021. https://doi.org/10.1161/CIRCRESAHA.113.302549

Sinno, H., Derakhchan, K., Libersan, D., Merhi, Y., Leung, T. K., & Nattel, S. (2003). Atrial ischemia promotes atrial fibrillation in dogs. *Circulation*, *107*, 1930–1936. https://doi.org/10.1161/01.CIR.0000058743.15215.03

Sipido, K. R., Volders, P. G., de Groot, S. H., Verdonck, F., Van de Werf, F., Wellens, H. J., & Vos, M. A. (2000). Enhanced Ca^{2+} release and Na/Ca exchange activity in hypertrophied canine ventricular myocytes: Potential link between contractile adaptation and arrhythmogenesis. *Circulation*, *102*, 2137–2144. https://doi.org/10.1161/01.cir.102.17.2137

Smoczyńska, A., Beekman, H. D., & Vos, M. A. (2019). The increment of short-term variability of repolarisation determines the severity of the imminent arrhythmic outcome. *Arrhythmia & Electrophysiology Review*, *8*, 166–172. https://doi.org/10.15420/aer.2019.16.2

Smoczyńska, A., Loen, V., Aranda, A., Beekman, H. D. M., Meine, M., & Vos, M. A. (2020). High-rate pacing guided by short-term variability of repolarization prevents imminent ventricular arrhythmias automatically by an internal cardioverter-defibrillator in the chronic atrioventricular block dog model. *Heart Rhythm*, *S1547-5271*, 30680–30689. https://doi.org/10.1016/j.hrthm.2020.07.023

Stams, T. R., Oros, A., van der Nagel, R., Beekman, J. D., Chamberlin, P., Dittrich, H. C., & Vos, M. A. (2011). Effects of K201 on repolarization and arrhythmogenesis in anesthetized chronic atrioventricular block dogs susceptible to dofetilide-induced torsade de pointes. *European Journal of Pharmacology*, *672*, 126–134. https://doi.org/10.1016/j.ejphar.2011.09.180

Starzl, T. E., & Gaertner, R. A. (1955). Chronic heart block in dogs; a method for producing experimental heart failure. *Circulation*, *12*, 259–270. https://doi.org/10.1161/01.cir.12.2.259

Sugiyama, A., Ishida, Y., Satoh, Y., Aoki, S., Hori, M., Akie, Y., Kobayashi, Y., & Hashimoto, K. (2002). Electrophysiological, anatomical and histological remodeling of the heart to AV block enhances susceptibility to arrhythmogenic effects of QT-prolonging drugs. *Japanese Journal of Pharmacology*, *88*, 341–350. https://doi.org/10.1254/jjp.88.341

Sugiyama, A., Satoh, Y., Shiina, H., Takeda, S., & Hashimoto, K. (2002). Torsadegenic action of the antipsychotic drug sulpiride assessed using in vivo canine models. *Journal of Cardiovascular Pharmacology*, *40*, 235–245. https://doi.org/10.1097/00005344-200208000-00009

Sugiyama, A., Satoh, Y., Takahara, A., Nakamura, Y., Shimizu-Sasamata, M., Sato, S., Miyata, K., & Hashimoto, K. (2003). Famotidine does not induce long QT syndrome: Experimental evidence from in vitro and in vivo test systems. *European Journal of Pharmacology*, *466*, 137–146. https://doi.org/10.1016/s0014-2999(03)01559-0

Takahara, A., Nakamura, Y., & Sugiyama, A. (2008). Beat-to-beat variability of repolarization differentiates the extent of torsadogenic potential of multi ion channel-blockers bepridil and amiodarone. *European Journal of Pharmacology*, *596*, 127–131. https://doi.org/10.1016/j.ejphar.2008.08.018

Takahara, A., Nakamura, Y., Wagatsuma, H., Aritomi, S., Nakayama, A., Satoh, Y., Akie, Y., & Sugiyama, A. (2009). Long-term blockade of L/N-

type Ca^{2+} channels by cilnidipine ameliorates repolarization abnormality of the canine hypertrophied heart. *British Journal of Pharmacology*, *158*, 1366–1374. https://doi.org/10.1111/j.1476-5381.2009.00407.x

Takahara, A., Sugiyama, A., Ishida, Y., Satoh, Y., Wang, K., Nakamura, Y., & Hashimoto, K. (2006). Long-term bradycardia caused by atrioventricular block can remodel the canine heart to detect the histamine H1 blocker terfenadine-induced torsades de pointes arrhythmias. *British Journal of Pharmacology*, *147*, 634–641. https://doi.org/10.1038/sj.bjp.0706493

Tang, X., Shi, L., Cui, X., Yu, Y., Qi, T., Chen, C., & Tang, X. (2017). Renal denervation decreases susceptibility of the heart to ventricular fibrillation in a canine model of chronic kidney disease. *Experimental Physiology*, *102*, 1414–1423. https://doi.org/10.1113/EP086370

Tawara, S. (1906). *Das Reizleitungssystem des Säugetierherzens*. Jena, Germany: Gustav Fischer Verlag.

Thomsen, M. B., Beekman, J. D., Attevelt, N. J., Takahara, A., Sugiyama, A., Chiba, K., & Vos, M. A. (2006). No proarrhythmic properties of the antibiotics Moxifloxacin or Azithromycin in anaesthetized dogs with chronic-AV block. *British Journal of Pharmacology*, *149*, 1039–1048. https://doi.org/10.1038/sj.bjp.0706900

Thomsen, M. B., Verduyn, S. C., Stengl, M., Beekman, J. D., de Pater, G., van Opstal, J., Volders, P. G., & Vos, M. A. (2004). Increased short-term variability of repolarization predicts *d*-sotalol-induced torsades de pointes in dogs. *Circulation*, *110*, 2453–2459. https://doi.org/10.1161/01.CIR.0000145162.64183.C8

Thomsen, M. B., Volders, P. G., Beekman, J. D., Matz, J., & Vos, M. A. (2006). Beat-to-beat variability of repolarization determines proarrhythmic outcome in dogs susceptible to drug-induced torsades de pointes. *Journal of the American College of Cardiology*, *48*, 1268–1276. https://doi.org/10.1016/j.jacc.2006.05.048

Thomsen, M. B., Volders, P. G., Stengl, M., Spätjens, R. L., Beekman, J. D., Bischoff, U., Kall, M. A., Frederiksen, K., Matz, J., & Vos, M. A. (2003). Electrophysiological safety of sertindole in dogs with normal and remodeled hearts. *The Journal of Pharmacology and Experimental Therapeutics*, *307*, 776–784. https://doi.org/10.1124/jpet.103.052753

Tibbitts, J. (2003). Issues related to the use of canines in toxicologic pathology—Issues with pharmacokinetics and metabolism. *Toxicologic Pathology*, *31*(Suppl), 17–24. https://doi.org/10.1080/01926230390174896

Torp-Pedersen, C., Møller, M., Bloch-Thomsen, P. E., Køber, L., Sandøe, E., Egstrup, K., Agner, E., Carlsen, J., Videbæk, J., Marchant, B., & Camm, A. J. (1999). Dofetilide in patients with congestive heart failure and left ventricular dysfunction. Danish Investigations of Arrhythmia and Mortality on Dofetilide Study Group. *New England Journal of Medicine*, *341*, 857–865. https://doi.org/10.1056/NEJM199909163411201

Tsuji, Y., Opthof, T., Yasui, K., Inden, Y., Takemura, H., Niwa, N., Lu, Z., Lee, J. K., Honjo, H., Kamiya, K., & Kodama, I. (2002). Ionic mechanisms of acquired QT prolongation and torsades de pointes in rabbits with chronic complete atrioventricular block. *Circulation*, *106*, 2012–2018. https://doi.org/10.1161/01.cir.0000031160.86313.24

Van Borren, M. M., Vos, M. A., Houtman, M. J., Antoons, G., & Ravesloot, J. H. (2013). Increased sarcolemmal Na^+/H^+ exchange activity in hypertrophied myocytes from dogs with chronic atrioventricular block. *Frontiers in Physiology*, *4*, 322. https://doi.org/10.3389/fphys.2013.00322

Van Opstal, J. M., Leunissen, J. D., Wellens, H. J., & Vos, M. A. (2001). Azimilide and dofetilide produce similar electrophysiological and proarrhythmic effects in a canine model of Torsade de Pointes arrhythmias. *European Journal of Pharmacology*, *412*, 67–76. https://doi.org/10.1016/s0014-2999(00)00943-2

Van Opstal, J. M., Schoenmakers, M., Verduyn, S. C., de Groot, S. H., Leunissen, J. D., van der Hulst, F. F., Molenschot, M. M., Wellens, H. J., & Vos, M. A. (2001). Chronic amiodarone evokes no torsade de pointes arrhythmias despite QT lengthening in an animal model of acquired long-QT syndrome. *Circulation*, *104*, 2722–2727. https://doi.org/10.1161/hc4701.099579

Van Weperen, V. Y. H., Bossu, A., & Vos, M. A. (2019). Point of view: Electrophysiological endpoints differ when comparing the mode of action of highly successful anti-arrhythmic drugs in the CAVB dog model with TdP. *Journal of Cardiovascular Pharmacology*, *74*, 499–507. https://doi.org/10.1097/FJC.0000000000000748

Vandersickel, N., Bossu, A., De Neve, J., Dunnink, A., Meijborg, V. M. F., van der Heyden, M. A., Beekman, J. D., De Bakker, J. M., Vos, M. A., & Panfilov, A. V. (2017). Short-lasting episodes of torsade de pointes in the chronic atrioventricular block dog model have a focal mechanism, while longer-lasting episodes are maintained by re-entry. *JACC: Clinical Electrophysiology*, *3*, 1565–1576. https://doi.org/10.1016/j.jacep.2017.06.016

Varkevisser, R., van der Heyden, M. A. G., Tieland, R. G., Beekman, J. D., & Vos, M. A. (2013). Vernakalant is devoid of proarrhythmic effects in the complete AV block dog model. *European Journal of Pharmacology*, *720*, 49–54. https://doi.org/10.1016/j.ejphar.2013.10.054

Varkevisser, R., Wijers, S. C., van der Heyden, M. A. G., Beekman, H. D. M., Meine, M. M., & Vos, M. A. (2012). Beat-to-beat variability of repolarization as a new biomarker for proarrhythmia in vivo. *Heart Rhythm*, *9*, 1718–1726. https://doi.org/10.1016/j.hrthm.2012.05.016

Varró, A., & Baczkó, I. (2011). Cardiac ventricular repolarization reserve: A principle for understanding drug-related proarrhythmic risk. *British Journal of Pharmacology*, *164*, 14–36. https://doi.org/10.1111/j.1476-5381.2011.01367.x

Verdonck, F., Volders, P. G., Vos, M. A., & Sipido, K. R. (2003). Increased Na^+ concentration and altered Na/K pump activity in hypertrophied canine ventricular cells. *Cardiovascular Research*, *57*, 1035–1043. https://doi.org/10.1016/s0008-6363(02)00734-4

Verduyn, S. C., Ramakers, C., Snoep, G., Leunissen, J. D., Wellens, H. J., & Vos, M. A. (2001). Time course of structural adaptations in chronic AV block dogs: Evidence for differential ventricular remodeling. *American Journal of Physiology. Heart and Circulatory Physiology*, *280*, H2882–H2890. https://doi.org/10.1152/ajpheart.2001.280.6.H2882

Verduyn, S. C., Vos, M. A., van der Zande, J., Kulcsàr, A., & Wellens, H. J. (1997). Further observations to elucidate the role of interventricular dispersion of repolarization and early afterdepolarizations in the genesis of acquired torsade de pointes arrhythmias: A comparison between almokalant and d-sotalol using the dog as its own control. *Journal of the American College of Cardiology*, *30*, 1575–1584. https://doi.org/10.1016/s0735-1097(97)00333-1

Volders, P. G., Sipido, K. R., Vos, M. A., Spätjens, R. L., Leunissen, J. D., Carmeliet, E., & Wellens, H. J. (1999). Downregulation of delayed rectifier K^+ currents in dogs with chronic complete atrioventricular block and acquired torsades de pointes. *Circulation*, *100*, 2455–2461. https://doi.org/10.1161/01.cir.100.24.2455

Vos, M. A., De Groot, S. H., Verduyn, S. C., Van der Zande, J., Leunissen, H. D., Cleutjens, J. P., Van Bilsen, M., Daemen, M. J., Schreuder, J. J., Allessie, M. A., & Wellens, H. J. (1998). Enhanced susceptibility for acquired torsade de pointes arrhythmias in the dog with chronic, complete AV block is related to cardiac hypertrophy and electrical remodeling. *Circulation*, *98*, 1125–1135. https://doi.org/10.1161/01.cir.98.11.1125

Vos, M. A., Gorgels, A. P., Drenth, J. P., Leunissen, J. D., & Wellens, H. J. (1989). Termination of ouabain-induced ventricular tachycardia by flunarizine in conscious dogs. *European Journal of Pharmacology*, *165*, 139–145. https://doi.org/10.1016/0014-2999(89)90780-2

Wang, Z., Pagé, P., & Nattel, S. (1992). Mechanism of flecainide's antiarrhythmic action in experimental atrial fibrillation. *Circulation Research*, *71*, 271–287. https://doi.org/10.1161/01.res.71.2.271

Watanabe, I., Okumura, Y., Ohkubo, K., Nagashima, K., Mano, H., Sonoda, K., Kofune, M., Kunimoto, S., Kasamaki, Y., & Hirayama, A. (2011). Effect of the ATP-sensitive K^+ channel opener nicorandil in a

canine model of proarrhythmia. *International Heart Journal*, *52*, 318–322. https://doi.org/10.1536/ihj.52.318

Watanabe, Y., Nakamura, Y., Cao, X., Ohara, H., Yamazaki, Y., Murayama, N., Sugiyama, Y., Izumi-Nakaseko, H., Ando, K., Yamazaki, H., & Sugiyama, A. (2015). Intravenous administration of apomorphine does not induce long QT syndrome: Experimental evidence from *in vivo* canine models. *Basic & Clinical Pharmacology & Toxicology*, *116*, 468–475. https://doi.org/10.1111/bcpt.12343

Weissenburger, J., Davy, J. M., Chézalviel, F., Ertzbischoff, O., Poirier, J. M., Engel, F., Lainee, P., Penin, E., Motte, G., & Cheymol, G. (1991). Arrhythmogenic activities of antiarrhythmic drugs in conscious hypokalemic dogs with atrioventricular block: Comparison between quinidine, lidocaine, flecainide, propranolol and sotalol. *Journal of Pharmacology and Experimental Therapeutics*, *259*, 871–883.

Werner, P., Raducha, M. G., Prociuk, U., Sleeper, M. M., Van Winkle, T. J., & Henthorn, P. S. (2008). A novel locus for dilated cardiomyopathy maps to canine chromosome 8. *Genomics*, *91*, 517–521. https://doi.org/10.1016/j.ygeno.2008.03.007

Wiggers, C. J. (1930). Studies of ventricular fibrillation caused by electric shock. II: Cinematographic and electrocardiographic observations of the natural process in the dogs heart. Its inhibition by potassium and the revival of coordinated beats by calcium. *American Heart Journal*, *5*, 351–365. https://doi.org/10.1016/S0002-8703(30)90334-1

Wiggers, C. J., & Wegria, R. (1940). Quantitative measurement of the the fibrillation thresholds of the mammalian ventricles with observations on the effect of procaine. *AJP-Legacy*, *131*, 296–308. https://doi.org/10.1152/ajplegacy.1940.131.2.296

Wiggers, C. J., Wegria, R., & Pinera, B. (1940). The effects of myocardial ischemia on the fibrillation threshold—The mechanism of spontaneous ventricular fibrillation following coronary occlusion. *AJP-Legacy*, *131*, 309–316. https://doi.org/10.1152/ajplegacy.1940.131.2.309

Wijers, S. C., Sprenkeler, D. J., Bossu, A., Dunnink, A., Beekman, J. D. M., Varkevisser, R., Hernández, A. A., Meine, M., & Vos, M. A. (2018). Beat-to-beat variations in activation-recovery interval derived from the right ventricular electrogram can monitor arrhythmic risk under anesthetic and awake conditions in the canine chronic atrioventricular block model. *Heart Rhythm*, *15*, 442–448. https://doi.org/10.1016/j.hrthm.2017.11.011

Wilburne, M., Surtshin, A., Rodbard, S., & Katz, L. N. (1947). Inhibition of paroxysmal ventricular tachycardia by atropine. *American Heart Journal*, *34*, 860–870. https://doi.org/10.1016/0002-8703(47)90150-6

Winckels, S. K. G., Thomsen, M. B., Oosterhoff, P., Oros, A., Beekman, J. D. M., Attevelt, N. J. M., Kretzers, L., & Vos, M. A. (2007). High-septal pacing reduces ventricular electrical remodeling and proarrhythmia in chronic atrioventricular block dogs. *Journal of the American College of Cardiology*, *50*, 906–913. https://doi.org/10.1016/j.jacc.2007.05.019

Yoshida, H., Sugiyama, A., Satoh, Y., Ishida, Y., Yoneyama, M., Kugiyama, K., & Hashimoto, K. (2002). Comparison of the in vivo electrophysiological and proarrhythmic effects of amiodarone with those of a selective class III drug, sematilide, using a canine chronic atrioventricular block model. *Circulation Journal*, *66*, 758–762. https://doi.org/10.1253/circj.66.758

Yu, G., Yu, Y., Li, Y. N., & Shu, R. (2010). Effect of periodontitis on susceptibility to atrial fibrillation in an animal model. *Journal of Electrocardiology*, *43*, 359–366. https://doi.org/10.1016/j.jelectrocard.2009.12.002

Zhu, Y., Hanafy, M. A., Killingsworth, C. R., Walcott, G. P., Young, M. E., & Pogwizd, S. M. (2014). Morning surge of ventricular arrhythmias in a new arrhythmogenic canine model of chronic heart failure is associated with attenuation of time-of-day dependence of heart rate and autonomic adaptation, and reduced cardiac chaos. *PLoS One*, *9*, e105379. https://doi.org/10.1371/journal.pone.0105379

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