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RESEARCH ARTICLE

Contribution of haemodynamic side effects and associated autonomic reflexes to ventricular arrhythmias triggering by torsadogenic hERG blocking drugs

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Background and Purpose: HERG blocking drugs known for their propensity to trigger Torsades de Pointes (TdP) were reported to induce a sympatho-vagal coactivation and to enhance High Frequency heart rate (HFHR) and QT oscillations (HFQT) in telemetric data. The present work aimed to characterize the underlying mechanism(s) leading to these autonomic changes.

Experimental Approach: Effects of 15 torsadogenic hERG blocking drugs (astemizole, chlorpromazine, cisapride, droperidol, ibutilide, dofetilide, haloperidol, moxifloxacin, pimozide, quinidine, risperidone, sotalol, sertindole, terfenadine, and thioridazine) were assessed by telemetry in beagle dogs. Haemodynamic effects on diastolic and systolic arterial pressure were analysed from the first doses causing QTc prolongation and/or HFQT oscillations enhancement. Autonomic control changes were analysed using the high frequency autonomic modulation (HFAM) model.

Key Results: Except for moxifloxacin and quinidine, all torsadogenic hERG blockers induced parasympathetic activation or sympatho-vagal coactivation combined with enhancement of HFQT oscillations. These autonomic effects result from reflex compensatory mechanisms in response to mild haemodynamic side effects. These haemodynamic mechanisms were characterized by transient HR acceleration during HF oscillations. A phenomenon of concealed QT prolongation was unmasked for several torsadogenic hERG blockers under β-adrenoceptor blockade with atenolol. Resulting enhancement of HFQT oscillations was shown to contribute directly to triggering dofetilide-induced ventricular arrhythmias.

Conclusion and Implications: This work supports for the first time a contribution of haemodynamic side properties to ventricular arrhythmias triggered by torsadogenic

Abbreviations: HFAM, high frequency autonomic modulation; HFQT, high frequency QT oscillations; VR, ventricular repolarisation.

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hERG blocking drugs. These haemodynamic side effects may constitute a second component of their arrhythmic profile, acting as a trigger alongside their intrinsic arrhythmogenic electrophysiological properties.

KEYWORDS
haemodynamic, hERG channel, HF oscillations, QTc prolongation, Torsades de Pointes

1 | INTRODUCTION

Torsades de Pointes (TdP) is a malignant form of ventricular arrhythmia that can degenerate into ventricular fibrillation and sudden cardiac death. TdP is commonly related to a lengthening of cardiac ventricular repolarisation (VR). Consequently, the increase in VR duration by small molecule drug candidates is a major issue since it is associated with the risk of TdP (Kannankeril et al., 2010). VR prolongation has been strongly linked to cardiac **hERG channel** blocking properties. Indeed, hERG channels are responsible for an inwardly rectifying outward potassium current, I_{KR}, that largely contributes to action potential repolarisation of ventricular cardiomyocytes. Despite the identification of this mechanism, prediction of the risk for QT prolongation remains uncertain during the preclinical development from hERG blocking properties and QT studies (Park et al., 2018). In addition to the lengthening of VR itself, the beat-to-beat variability of VR (BVR) also plays a major role in TdP (Lengyel et al., 2007; Thomsen et al., 2004). In sinus rhythm, the greatest contributor to BVR is the parasympathetic nervous system through rate-dependent mechanisms. Indeed, rhythmic vagal discharges in the High Frequency (HF) band (>0.1 Hz) are responsible for large beat-to-beat HR changes, causing in turn an important beat-to-beat rate dependent adaptation of VR duration and the QT interval. Interestingly, both magnitudes of HF oscillations of heart rate (HFHR) and HF oscillations of the QT interval (HFQT) were increased by several hERG blockers responsible for TdP in dogs (Champéroux et al., 2016), and their suppression by a ganglioplegic agent fully prevents **dofetilide**-induced TdP in cynomolgus monkeys (Champéroux et al., 2015). We also showed that increases in HFQT oscillations induced by hERG blockers may result from a sympathetic activation concomitant with HF parasympathetic oscillations (Champéroux et al., 2016). Altogether, using a new method of analysis of RR time-series, named HFAM (for high-frequency autonomic modulation), we were able to characterize a particular state of the autonomic control induced by hERG blockers, named S2 oscillations which originate from, and reflect, a sympatho-vagal coactivation (Champéroux et al., 2018). However, the mechanism by which torsadogenic hERG blockers causes this coactivation is still unclear. Parasympathetic and/or sympathetic activation often results from reflex compensatory mechanisms in response to haemodynamic changes (Dampney, 2016). So, we focused on possible haemodynamic effects of hERG blocking drugs and their underlying consequences on HF oscillations using a large set of torsadogenic hERG blockers.

What is already known

- Enhancement of HFQT oscillations by dofetilide contributes to triggering of Torsades de Pointes.
- Enhancement of HFQT oscillations by torsadogenic drugs reflects a sympatho-vagal coactivation.

What does this study add

- Enhancement of HFQT oscillations and sympatho-vagal coactivation induced by torsadogenic drugs results from haemodynamic effects.

What is the clinical significance

- Haemodynamic side effects of torsadogenic hERG-blocking drugs can trigger ventricular arrhythmias through associated autonomic reflexes.
- The haemodynamic component opens new avenues for drug risk stratification in precision medicine.

2 | METHODS

Torsadogenic drugs are defined as drugs in clinical reports of Torsades de Pointes (Redfern et al., 2003). Effects of 15 torsadogenic hERG blocking drugs were characterized from the first doses causing either QTc prolongation and/or increase in HFQT oscillations, also called "early" doses throughout the manuscript. These drugs reported as torsadogenic are: **astemizole**, **chlorpromazine**, **cisapride**, dofetilide, **droperidol**, **haloperidol**, **ibutilide**, **moxifloxacin**, **pimozide**, **quinidine**, **risperidone**, **sotalol**, **sertindole**, **terfenadine**, and **thioridazine**.

2.1 | Telemetric recordings

Twenty-four-hour telemetric ECG and BP recordings were collected in beagle dogs. All animal experiments were subjected to ethical

review (Ethics Committee no. CEEA-111) according to European directive 2010/63/UE on animal welfare. Reporting of experiments follows the ARRIVE guidelines (Kilkenny et al., 2010; McGrath & Lilley, 2015). This work complies with all requirements of the *BJP's* Declaration on Experimental Design and Analysis. In accordance with the 3Rs encouraging the reduction in the number of animals used for experimental research, experiments already recorded in the internal ERBC database from 2008 until mid-2020 were re-analysed for this work.

All animal experiments were conducted in the following conditions described thereafter. Adult (three males and three females per group for former studies or six males per group for recent studies due to logistic reasons linked to group housing) beagle dogs (10–15 kg, 8–24 months, CEDS, Mezilles, France) were fitted with radio telemetry transmitters (TL11M2D70PCT, L11 or M11 models, Data Sciences International, Saint Paul, USA). Dogs were premedicated with acetylpromazine (0.05 $mg \cdot kg^{-1}$, s.c.) and **buprenorphine** (0.01 $mg \cdot kg^{-1}$, s.c.). Anaesthesia was induced by **thiopental** (15–20 $mg \cdot kg^{-1}$, i.v.) and then maintained with **isoflurane** (0.5%–1.5% in oxygen). After left thoracotomy, one electrode was sutured directly to the left ventricular epicardium near the apex while the second electrode was sutured to the pericardium above the right atrium, to approximate a limb Lead II ECG. The BP sensor was introduced via the femoral artery up to the caudal portion of the abdominal aorta. Analgesic treatment with buprenorphine/meloxicam was continued for a minimum of 2 days to alleviate any post-operative pain. A minimum period of 3 weeks was allowed for recovery from the surgery. For experiments conducted for the present study, animals were housed in pens in groups of two to four animals at maximum and with playing tools. Environmental parameters were recorded continuously and maintained within a fixed-range room temperature of 15–21°C, at 45%–65% relative humidity. The artificial day/night cycle was 12 h light and 12 h darkness with lights on at 07:30 am. Drinking water was provided ad libitum. Solid diet (300 g per animal) was given daily in the morning. All dosing with drugs was performed between 2:00 and 3:30 PM. BP and ECGs were recorded continuously for a minimum of 2 h before dosing up to 24 h post dose. Animals served as their own control according to cross-over design with a washout period of 48 h at minimum up to 1 week between dosing sessions, depending on available information about terminal elimination half-life. The "drug combined with **atenolol**" session was included in the cross-over design. In case of long terminal elimination half-life (e.g., sotalol and haloperidol), an ascending dose design was followed. In that case, two additional sessions, vehicle versus drug combined with atenolol (as a **β_1-adrenoceptor** blocker) were performed on the same animals, as far as possible. In this case, the two-vehicle sessions were merged for the statistical analysis. This latter design was followed for the experiment dofetilide combined with **hexamethonium**. Experiments dofetilide versus dofetilide combined with **atropine** or with atenolol were performed following a cross-over design. ECG and BP signals were recorded at a sampling rate of 500 Hz, using ART™ acquisition software (release 4.33, Data Sciences International, St Paul, MN, USA) or Ponemah™ (release 6.33, Data Sciences International, St Paul, MN, USA).

2.2 | Beat-to-beat analysis and QT correction

Beat-to-beat heart rate (HR), mean (MAP), diastolic (DAP), and systolic (SAP) arterial pressures were calculated from the BP signal using a software developed in RPL (RS/1 programming language, RS/1 release 6.3, Applied Materials). RR interval (ms) was derived from beat-to-beat HR values: RR = 60/HR*1000. HR was derived from the BP signal to minimize risk of missed beats that could markedly bias the HFAM model. Indeed, except using epicardial ECG electrodes placement, this risk should be considered as high with subcutaneous placement of ECG electrodes (the most popular placement). Furthermore, minimizing this risk allows full automation of calculations for the HFAM model. The calculations of the HFAM model from the BP signal have been validated and produce identical results to those obtained from epicardial ECG. QT interval and all derived parameters (QTc, HFQT) were calculated from the ECG signal using a software developed in RPL (RS/1 programming language, RS/1 release 6.3, Applied Materials). QT interval was corrected by HR using the Holzgrefe's probabilistic QT correction method (Holzgrefe et al., 2014). The Holzgrefe's formula is the following: $QTc = 10^{(\log QT - \beta * [\log RR - \log RRref])}$. This method requires many beats for each discrete QTc value ($n > 250$ in dogs). All beats collected for 1-h sequences were included in the calculation of QTc values. Mean values of all parameters were calculated within discrete 10-s sequences (binning) to match the HFAM model.

2.3 | HF oscillations

The term "HF oscillations" for high frequency (>0.1 Hz) oscillations refers to the largest magnitude of beat-to-beat heart rate (HFHR), heart period (HFRR), and QT interval (HFQT) changes measured within discrete 10-s sequences (Champéroux et al., 2015, 2016, 2018). Examples of large HFHR and HFQT oscillations after pharmacological dosing with thioridazine and dofetilide are done in Figure 1.

2.4 | HFAM model

The HFAM model was demonstrated to provide reliable modelling of the autonomic balance of the HR control in beagle dogs, cynomolgus monkeys, and humans (Champéroux et al., 2018). This model is based on the principle that the magnitude of HFRR oscillations measured within discrete 10-s sequences are mainly dependent on the parasympathetic system while the magnitude of HFHR oscillations are dependent on both parasympathetic and sympathetic nervous systems. This model requires calculations of $HFHR_{ref}$ and $HFRR_{ref}$ values

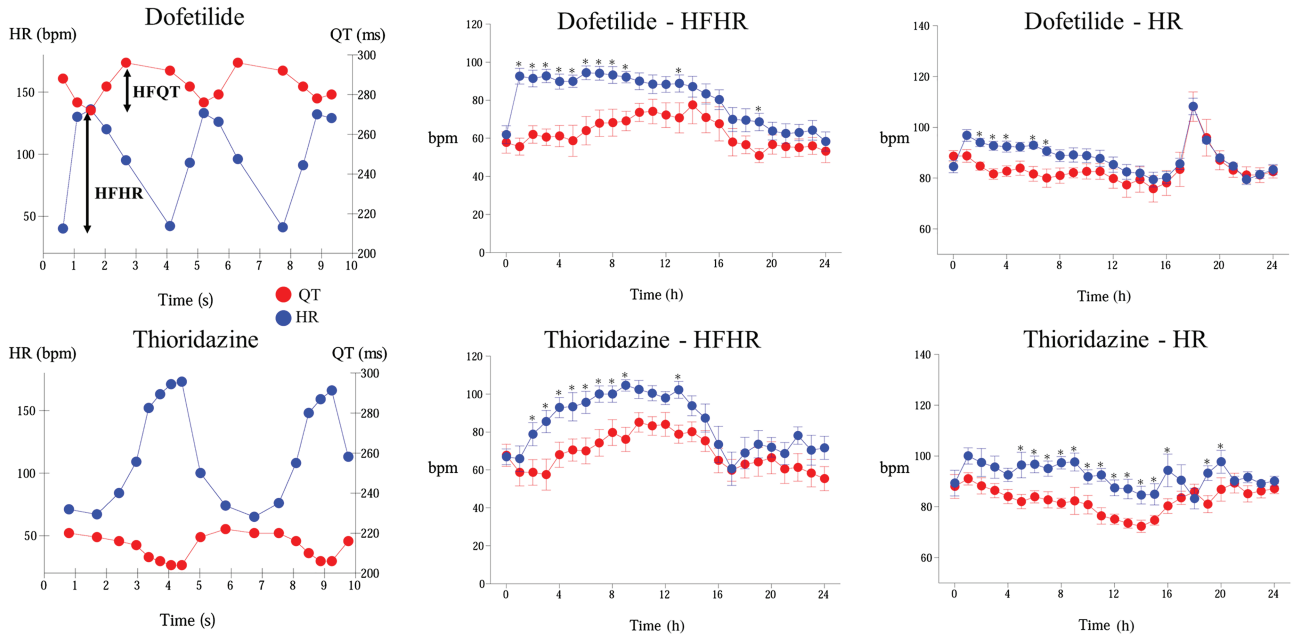

FIGURE 1 Left: Typical examples of HFHR oscillations (blue line) and related beat-to-beat HFQT oscillations (red line) induced by dofetilide (0.1 mg·kg^{-1}, p.o.) and thioridazine (1.5 mg·kg^{-1}, p.o.) in beagle dogs. HFQT oscillations are visible in the absence of QT prolongation for thioridazine and in the presence of QT prolongation for dofetilide. Middle and right: Effects of dofetilide (1 mg·kg^{-1}, p.o.) and thioridazine (1.5 mg·kg^{-1}, p.o.) on HFHR and mean HR. vehicle: Red filled circles, drug alone: Blue filled circles. Data are presented as mean values ± SEM ($n = 6$, $^*P \leq 0.05$, when compared with vehicle)

individually for each animal during a free-treatment 24-h recording session preceding each experiment. $HFHR_{ref}$ and $HFRR_{ref}$ values are the mean values of HFHR and HFRR oscillations calculated over the entire circadian period. The HFAM ratio is an index of the sympatho-vagal balance (Champéroux et al., 2018). It requires normalization of HFHR and HFRR oscillations as follows:

$HFHRN = HFHR/HFHR_{ref}$ in normalized units
$HFRRN = HFRR/HFRR_{ref}$ in normalized units

Then, the HFAM index is calculated from the following ratio: $HFAM = HFHRN/HFRRN$.

The HFAM model allows differentiation of three states of the autonomic control named S1, S2, and S3. Discrete algorithms were designed to automatically identify each state within discrete 10-s sequences:

S1: HFAM ≤ 1.
S2: HFAM > 1 and HFHRN > 1.
S3: HFAM > 1 and HFHRN < 1.

According to this model, S1 oscillations was shown to correspond to discrete 10-s sequences where the parasympathetic system is predominant. They are characterized by well-shaped and stable beat-to-beat HR oscillations. The S2 oscillations meanwhile, correspond to discrete 10-s sequences where both the parasympathetic system and the sympathetic system are co-activated. They were characterized by larger beat-to-beat HR oscillations (usually >80 bpm in dogs) than S1 oscillations and by the presence of marked transient sequences of HR accelerations after the sequences of deceleration inside high frequency HR oscillations. Finally, S3 oscillations were shown to correspond to discrete 10-s sequences where the magnitude of HR oscillations is blunted, that is, with a smoothed aspect and reduced magnitude (usually <30–40 bpm in dogs). They reflect reciprocal parasympathetic withdrawal due to sympathetic activation.

In ambulatory conditions, high frequency rhythms are not stationary especially during the diurnal phase of the circadian cycle. The HFAM model provides modelling of the statistical distribution of these three simply defined states of the autonomic control calculated over short 10-s sequences. Accordingly, S1, S2, and S3 outcomes are expressed in proportion in % per hour. In 24-h safety pharmacology studies, this time interval of 1 h is the most appropriate to assess drug effect at steady state since calculated from numerous 10-s sequences ($n = 360$/h). Only the HFAM index can be considered as directly related to the magnitude of parasympathetic or sympathetic activities (Champéroux et al., 2018).

2.5 | Arrhythmias and conduction troubles analysis

Arrhythmias and conduction troubles analyses were performed following the Lambeth conventions (Curtis et al., 2013). Ten-second sequences with high probability of arrhythmias or conduction troubles were preselected over the whole post-dosing 24 h period using an internal software developed in RPL, validated according to GLP (Good Laboratory Practices), from epicardial ECG signal having advantage to

limit electric noise and the presence of isoelectric line fluctuations (Holzgrefe et al., 2007). This quality level makes it possible to fully automate the detection of cardiac arrhythmias and conduction troubles with high resolution. Probability to find treatment-related arrhythmias and conduction troubles in treated sessions was determined from our internal database which includes results of the analysis 24-h recordings from 655 control sessions. Qualification of arrhythmias was performed manually.

2.6 | Statistical procedures

The data and statistical analysis comply with the recommendations of the *British Journal of Pharmacology* on experimental design and analysis in pharmacology. Comparisons between groups were performed using an analysis of variance for repeated measures and Dunnett's test. The level of probability (P) deemed to constitute the threshold for statistical significance was fixed to $P \leq 0.05$. Results are expressed as mean values ± SEM. The exact group size (n) is provided in legends of tables and figures. "n" values refer to independent animals or subjects and not replicates. Statistics were performed using RS/1 release 6.3, Applied Materials. Operators for animal experiments and data analysts were not blinded. This work does not contain any subjective observation or analyses that would justify blinding. Furthermore, all calculations were made by using fully automated computer procedures, validated according to GLP (Good Laboratory Practices), from beat-to-beat analysis up to statistics. In addition, subgroups of torsadogenic drugs with common profiles were determined analysis of principal components and from hierarchical cluster analysis using the WARD method in R 4.1.3.

2.7 | Materials, drugs and choice of doses

Molecules were purchased from Abcam Biochemicals (Cambridge, United Kingdom), Biotrend (Chemikalien GmbH, Koln, Germany), Carbosynth Ltd (Berkshire, United Kingdom), Clinisciences (Nanterre, France), Sequoia Research Product Ltd (Pangbourne, United Kingdom), and Sigma-Aldrich (Saint Quentin, France). The list of drugs including suppliers, vehicles, and volumes of administration depending on dose levels and route administration are available as raw data. Doses were selected from preliminary trials to determine the minimum effective dose causing either QTc prolongation, increase in HFQT oscillations or change in autonomic control.

2.8 | Data availability

Raw data, that is, the list of drugs including suppliers, vehicles and volumes of administration, graphs with mean curves and statistics for each molecule and parameter, graphs for each molecule combined with atenolol, summaries of analysis of arrhythmias/conduction troubles over 24-h post-dosing period for each torsadogenic hERG blockers given alone, individual time distributions of arrhythmias induced by dofetilide at a high dose alone or in combination with atropine or hexamethonium are available at Figshare: doi: 10.6084/m9.figshare.19322975.

2.9 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/22 (Alexander, Christopoulos, et al., 2021; Alexander, Kelly, et al., 2021; Alexander, Mathie, et al., 2021).

3 | RESULTS

Most of tested torsadogenic hERG blockers showed various haemodynamic and electrophysiologic profiles at early doses, causing QTc prolongation and/or HFQT oscillations enhancement. Results are reported in bar graphs showing peaks of effects of each drug to facilitate comparisons (Figure 2). Peaks of effects were found within the first 12 h post-dosing except for cisapride, droperidol, and terfenadine which showed delayed effects.

3.1 | Electrophysiological, autonomic, and haemodynamic effects

Two examples of large HFHR and HFQT oscillations are shown for dofetilide and thioridazine (Figure 1). Both drugs caused a large increase in HFHR oscillations. They also induced mild increases in mean HR. This increase in mean HR is due to transient sequences of high beat-to-beat HR visible during HF oscillations. These two examples demonstrate that increases in HF oscillations magnitude can be independent of a lower mean HR level despite the vagal origin of HF oscillations. Indeed, counterintuitively, 12 torsadogenic hERG blockers showed increases in mean HR associated with enhanced HFHR oscillations for 10 of them (Figure 2). These 12 molecules showed enhanced HFQT. According to the HFAM model, the proportion of S1 and/or S2 oscillations reflecting parasympathetic predominance and S2 sympatho-vagal oscillations respectively was increased for 12 molecules. Reciprocally, proportion of S3 oscillations reflecting parasympathetic withdrawal (i.e., smoothed vagal oscillations with low magnitude) was largely decreased for 12 molecules. Only, 10 out the 15 torsadogenic hERG blocking drugs exhibited QTc prolongation. Four molecules increased MAP, DAP, and SAP; three others induced only mild decreases in SAP; and three others caused only a decrease in minimum DAP (DAPmin) during HF oscillations (Figure 2). Both principal components analysis and hierarchical cluster analysis allow identification of four subgroups showing common haemodynamic, autonomic, and electrophysiological features and profiles (Figure 3).

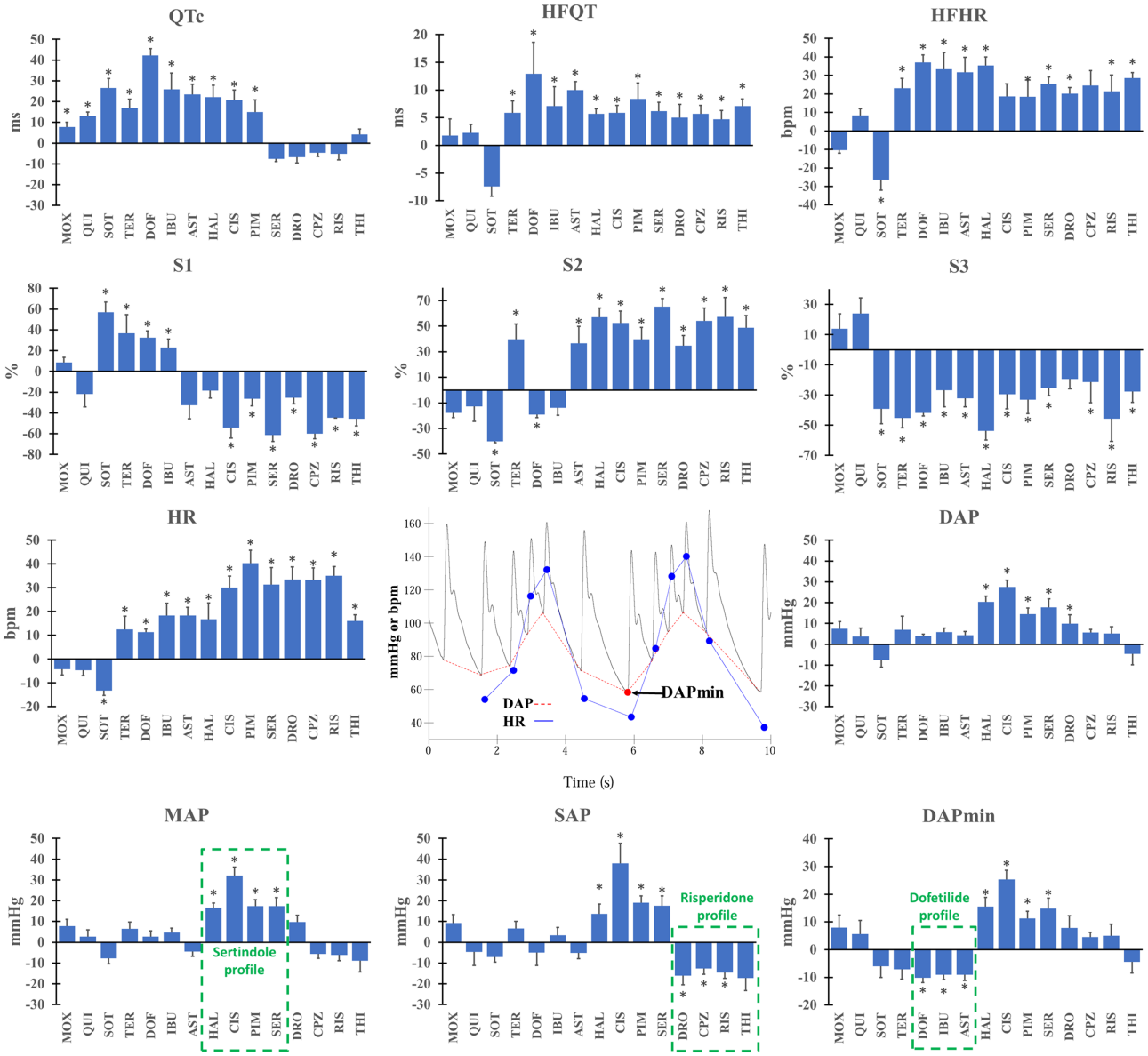

FIGURE 2 Effects of moxifloxacin (MOX, 10 mg·kg^{-1}, p.o.), quinidine (QUI, 10 mg·kg^{-1}, p.o.), sotalol (SOT, 10 mg·kg^{-1}, p.o.), dofetilide (DOF, 0.1 mg·kg^{-1}, p.o.), terfenadine (TER, 30 mg·kg^{-1}, p.o.), ibutilide (IBU, 1 mg·kg^{-1}, i.v.), astemizole (AST, 1 mg·kg^{-1}, i.v.), haloperidol (HAL, 3 mg·kg^{-1}, p.o.), cisapride (CIS, 6 mg·kg^{-1}, p.o.), pimozide (PIM, 1 mg·kg^{-1}, i.v.), sertindole (SER, 1 mg·kg^{-1}, i.v.), droperidol (DRO, 3 mg·kg^{-1}, i.v.), chlorpromazine (CPZ, 1 mg·kg^{-1}, i.v.), risperidone (RIS, 1 mg·kg^{-1}, i.v.) and thioridazine (THI, 1.5 mg·kg^{-1}, p.o.) on QTc interval, HFQT and HFHR oscillations, proportions (% per hour) of S1 parasympathetic predominant, S2 sympatho-vagal and S3 parasympathetic withdrawal oscillations, mean HR, SAP, MAP, DAP and DAPmin values. An example of large HFHR oscillations with superimposed BP signal is provided to depict DAP oscillations and calculation of the DAPmin value during HF oscillations. Bar graphs report maximum effect at peak calculated as variation in relation to vehicle. Specific haemodynamic effects of dofetilide, sertindole, and risperidone profiles are highlighted by dotted green boxes. Data are presented as mean values ± SEM ($n = 6$, $^*P \leq 0.05$, when compared with vehicle)

These profiles can be depicted by taking four molecules only as reference examples.

Quinidine: This drug caused a mild QTc prolongation without any sign of mean HR and HFQT oscillations changes (Figure 4). The mild QTc prolongation was not associated with any autonomic and haemodynamic changes (Figure 2). Moxifloxacin also shared this profile (Figure 2). The profile of sotalol is close to that of quinidine and moxifloxacin. Indeed, this drug did not show an increase in HR or enhancement of HFHR and HFQT oscillations in agreement with its intrinsic β-adrenoceptor blocking properties (Figure 2). However, sotalol induced large QTc prolongation at early doses as found with dofetilide and other drugs in the dofetilide subgroup.

Dofetilide: This drug caused large QTc prolongation from the earliest dose (0.1 mg·kg^{-1}, p.o.) associated with enhanced HFQT oscillations (Figure 4). No change in mean DAP and SAP was found (Figure 2). The parasympathetic system was the predominant autonomic state (S1 oscillations), suggesting a reflex vagal activation following drug

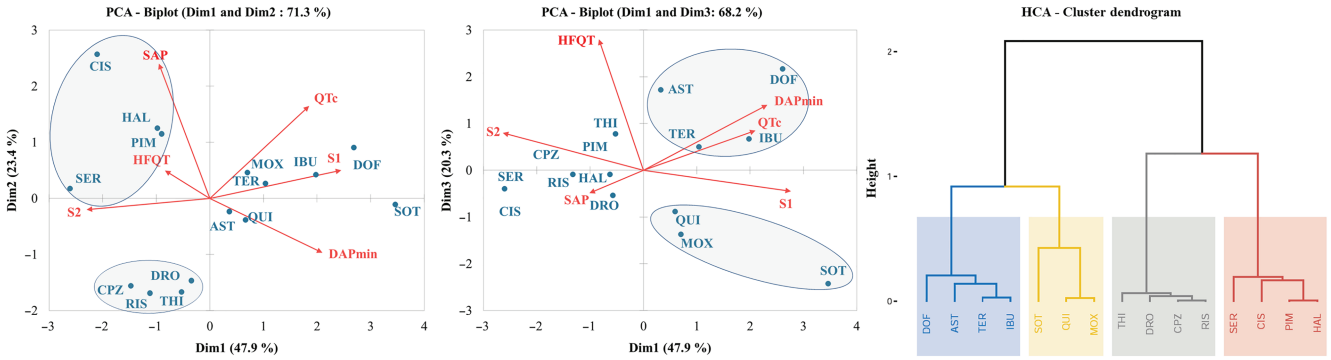

FIGURE 3 The three first components of the principal components analysis (PCA) explain more than 90% of the variance of the model reduced to six variables: SAP, decrease in DAPmin, QTc, S1, S2, and HFQT at peaks of effects. Axes of the two first components (Dim1 and Dim2) allow to identify two first subgroups, SAP being the most contributing variable (58%) of the second component (Dim2). The first and the third components (Dim1 and Dim3) allow to identify two other subgroups, HFQT being the most contributing variable (69%) of the third component (Dim3). Hierarchical clustering analysis (HCA - cluster dendrogram) shows the same classification. AST (astemizole, 1 mg·kg^{-1}, i.v.), CIS (cisapride, 6 mg·kg^{-1}, p.o.), chlorpromazine (CPZ, 1 mg·kg^{-1}, i.v.), DOF (dofetilide, 0.1 mg·kg^{-1}, p.o.), DRO (droperidol, 3 mg·kg^{-1}, i.v.), HAL (haloperidol, 3 mg·kg^{-1}, p.o.), IBU (ibutilide, 1 mg·kg^{-1}, i.v.), MOX (moxifloxacin, 10 mg·kg^{-1}, p.o.), PIM (pimozide, 1 mg·kg^{-1}, i.v.), QUI (quinidine, 10 mg·kg^{-1}, p.o.), RIS (risperidone, 1 mg·kg^{-1}, i.v.), SER (sertindole, 1 mg·kg^{-1}, i.v.), SOT (sotalol, 10 mg·kg^{-1}, p.o.), TER (terfenadine, 30 mg·kg^{-1}, p.o.), and THI (thioridazine, 1.5 mg·kg^{-1}, p.o.)

dosing. Transient decrease in DAP during HF oscillations (DAPmin) is observed at the termination of the largest RR pause during HF oscillations (Figure 2). DAPmin values were lower with dodetilide. This was the only visible haemodynamic change observed with dofetilide (Figure 2). These low DAPmin levels were followed by transient sequences of HR acceleration over two to three beats likely to maintain unchanged both DAP and SAP values and compensate the haemodynamic mechanism responsible for the vagal activation. Ibutilide is the only other molecule fully sharing this profile (Figures 2). Indeed, while astemizole also decreased DAPmin, it induced a sympatho-vagal S2 coactivation (Figure 2). Terfenadine also shares several features with these three molecules (Figure 2) but it showed delayed and mitigated QTc prolongation.

Sertindole: This drug (Figure 4) shares with haloperidol, cisapride and pimozide a profile characterized by an increase in MAP, DAP and SAP, predominant sympatho-vagal coactivation (S2) and enhancement of HFQT oscillations (Figure 2) following administration. These four molecules induced an increase in mean HR. Haloperidol is the only drug causing QTc prolongation from an early dose.

Risperidone: This drug (Figure 4) shares with droperidol, chlorpromazine and thioridazine a profile characterized by a mild decrease in SAP, predominant sympatho-vagal coactivation (S2) and enhancement of HFQT oscillations (Figure 2). These four molecules also caused an increase in mean HR. None of these drugs caused directly visible QTc prolongation at early doses.

3.2 | Concealed QTc prolongation

From signal analysis, we observed that six drugs induced concealed QTc prolongation. The QTc lengthening was unmasked when drugs were combined with atenolol, a β-adrenoceptor blocking drug (Figure 5). When given alone, atenolol was devoid of any electrophysiologic and haemodynamic effect (available in raw data only).

3.3 | The arrhythmic component versus haemodynamic/autonomic component of torsadogenic hERG blockers

We first report arrhythmias detected for the 15 torsadogenic hERG blockers at early doses causing QTc prolongation and/or HFQT oscillations enhancement. Figure 6 reports a summary of the main findings. We observed that about half of torsadogenic hERG blockers did not cause treatment-related ventricular arrhythmias or conduction troubles. The other half caused both ventricular arrhythmias, mainly ectopic and premature ventricular beats, and conduction troubles characterized by left or right bundle branch blocks. Half of the animals or less did not develop treatment-related ventricular arrhythmias or conduction troubles depending on the molecule. In addition, the maximum incidence of treatment-related ventricular arrhythmias (per approximately 100,000 beats per 24-h session) reported in the Figure 6 indicates it was very low in most cases except for terfenadine in one animal for ventricular arrhythmias and for dofetilide, ibutilide and astemizole for bundle branch blocks. In summary, the probability of triggering ventricular arrhythmias or conduction troubles remains very low in animals treated with torsadogenic hERG blockers at early doses causing QTc prolongation and/or HFQT oscillations enhancement. The achievement of more reproducible ventricular arrhythmias in all animals with a higher incidence by the oral route was possible with dofetilide at a dose of 1 mg·kg^{-1} and by selection of animals for their sensitivity to arrhythmic effects of dofetilide. Figure 7 shows the QT prolongation and the increase in HFHR and HFQT oscillations

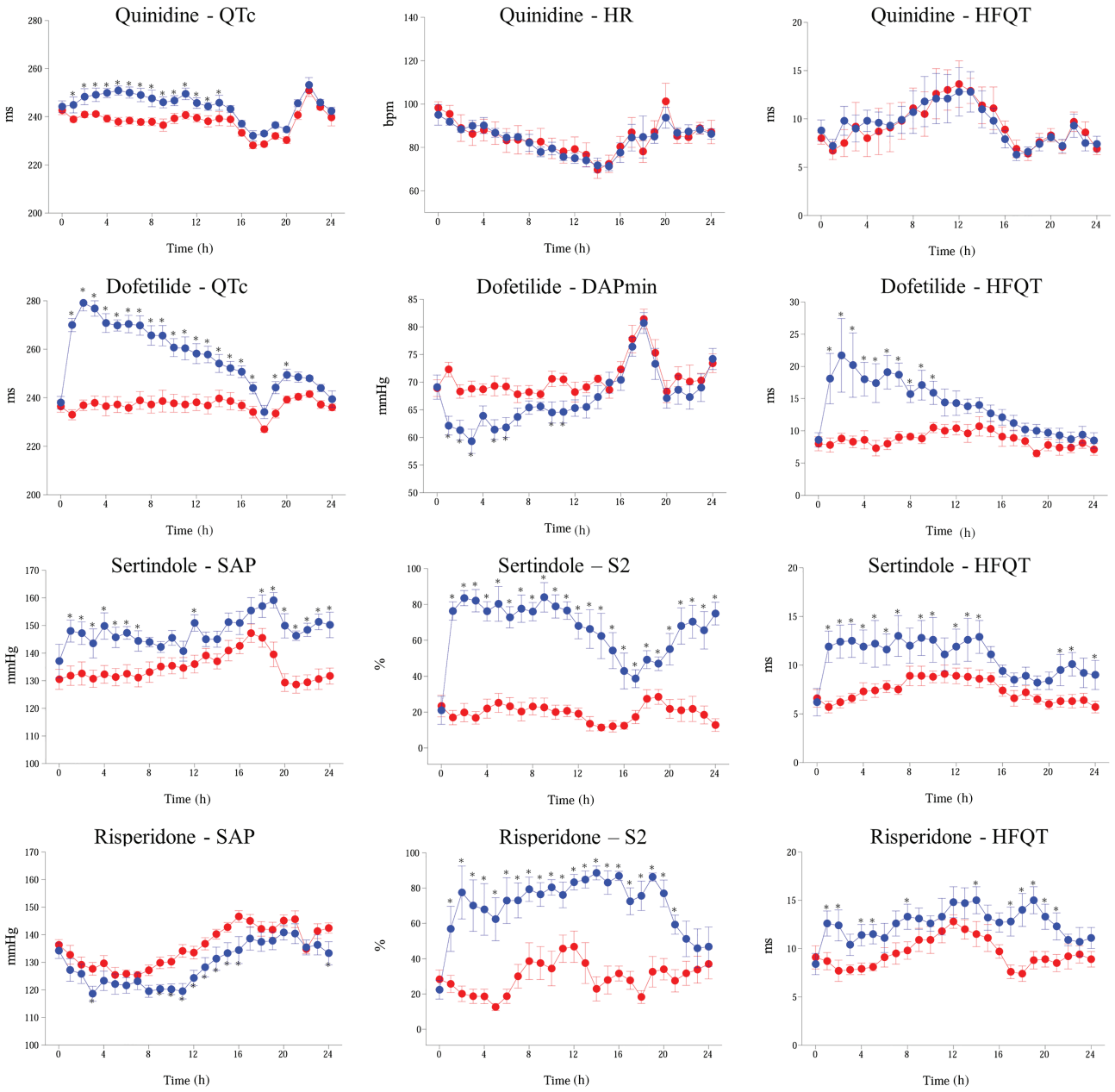

FIGURE 4 Four drugs were chosen to depict the various electrophysiologic, autonomic, and haemodynamic profiles of the 15 torsadogenic hERG blockers. "Quinidine profile": Characterized by a mild QTc prolongation and the absence of any autonomic effects (mean HR and HFQT oscillations) due the absence of haemodynamic effects. "Dofetilide profile": Large QTc prolongation associated with decrease in DAPmin during HF oscillations and enhancement of HFQT oscillations. "Sertindole profile": Increase in SAP combined with enhancement of sympatho-vagal S2 and HFQT oscillations. "Risperidone profile": Decrease in SAP combined with enhancement of sympatho-vagal S2 and HFQT oscillations. "Quinidine: 10 mg·kg^{-1}, p.o. Dofetilide: 0.1 mg·kg^{-1}, p.o. Sertindole: 1 mg·kg^{-1}, i.v., Thioridazine: 1.5 mg·kg^{-1}, p.o. Vehicle: red filled circles, drug alone: blue filled circles. Data are presented as mean values ± SEM ($n = 6$, *$P \leq 0.05$, when compared with vehicle)"

induced by dofetilide at a high dose (1 mg·kg^{-1}, p.o.) alone or under autonomic blockers (atropine and hexamethonium). It must be pointed out that the magnitude of QTc prolongation and HFHR/HFQT oscillations are similar at peak to that found at the lower dose of 0.1 mg·kg^{-1} (Figures 1 and 2). The kinetics of changes of QTc prolongation, HFHR and HFQT oscillations with vehicle, with dofetilide alone, and with dofetilide under atropine or hexamethonium are shown in Figure 7. Atropine is a **muscarinic receptor** antagonist which selectively blocks the parasympathetic control of HR at the sinus node. Hexamethonium is a ganglioplegic agent which causes a full blockade of the autonomic sympathetic and parasympathetic control. Both agents fully suppress HF oscillations. The mean times of onset of the first arrhythmias and the mean times of observation of the last arrhythmias were superimposed in all graphs. With dofetilide alone, ventricular ectopic or premature beats were rapidly observed because the QT value and HF oscillations increased after dosing. Indeed, ventricular arrhythmias

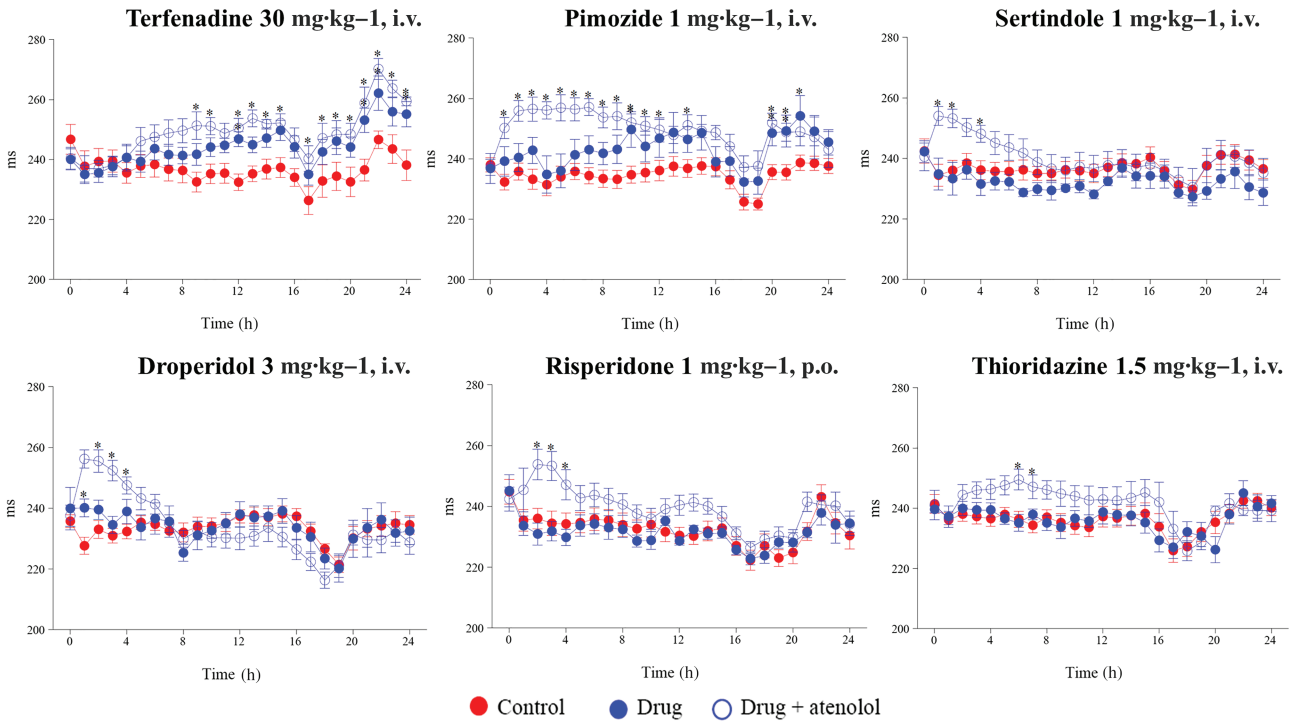

FIGURE 5 Examples of concealed QTc prolongation. In all these typical examples, drugs alone did not induce any visible QTc prolongation. QTc prolongation induced by these hERG channel blockers were unmasked in the presence of the β-blocker atenolol (1 mg>/kg/i.v.). Vehicle: Red filled circles, drug alone: Blue filled circles, drug combined with atenolol: Blue empty circles. Data are presented as mean values ± SEM ($n = 6$, *: $P \leq 0.05$, when compared with vehicle)

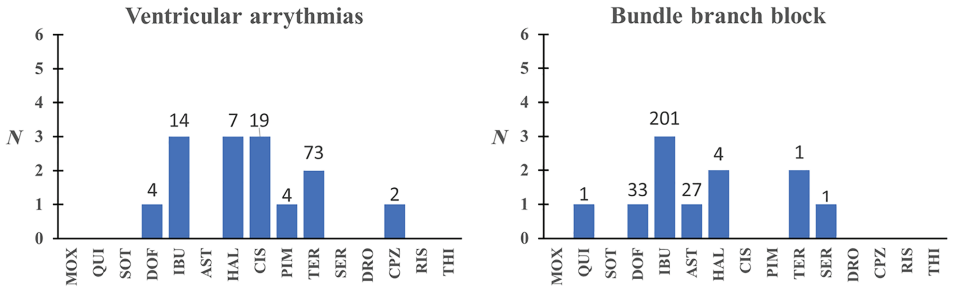

FIGURE 6 Summary bar graphs reporting the number of animals in each group showing ventricular arrhythmias (left) and bundle branch blocks (right) for the 15 torsadogenic hERG blocking drugs. Above each bar is mentioned the number of arrhythmic events or BBB detected over the 24-h post dosing period in the animal showing the greatest number of events

started on average at 1.4 ± 0.7 h after dofetilide dosing when given alone. Under atropine, dofetilide-induced ventricular arrhythmias were completely suppressed in parallel with HFHR/HFHQT oscillations and autonomic blockade but not in parallel with QT interval prolongation. Indeed, the return of QT to values close to those observed with dofetilide alone was observed 5 h post dosing while the onset of ventricular arrhythmias was delayed to 9 ± 1.1 h (Figure 7) and did not reappear until the HFHR and HFQT oscillations had returned to the same magnitude as with dofetilide alone. Under autonomic blockade by hexamethonium, the onset of ventricular arrhythmias was delayed to 6 ± 0.3 h under hexamethonium, that is, for the duration of the suppression of HF oscillations.

4 | DISCUSSION

This work was performed on a set of 15 hERG blocking torsadogenic drugs including 9 of the 10 most potent hERG blockers in the Kramer list (Kramer et al., 2013). This study aimed to characterize the underlying mechanisms responsible for sympatho-vagal coactivation and enhancement of HFHR and HFQT oscillations reported for several torsadogenic hERG blockers (Champéroux et al., 2016, 2018). This characterization is highly relevant since it can open new avenues in the understanding of the pro-arrhythmic effects of torsadogenic drugs. The initial hypothesis was that these autonomic changes might result from reflex compensatory mechanisms in response to haemodynamic

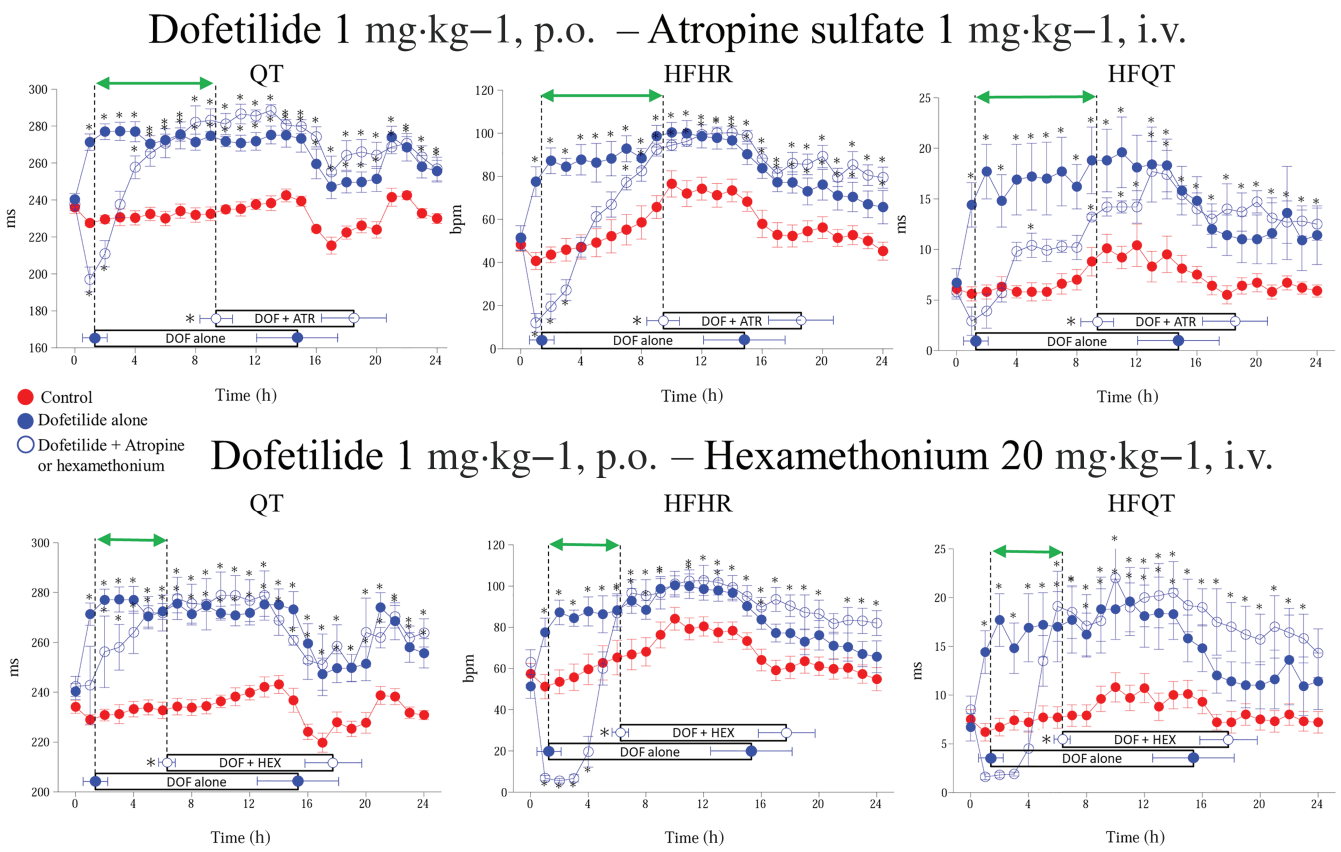

FIGURE 7 Upper panel: Effect of dofetilide (1 mg·kg^{-1}, p.o.) alone or in combination with atropine sulphate (1 mg·kg^{-1}, i.v.) on QT interval, HFHR oscillations and HFQT oscillations. Lower panel: Effect of dofetilide (1 mg·kg^{-1}, p.o.) alone or in combination with hexamethonium (20 mg·kg^{-1}, i.v.) on QT interval, HFHR oscillations, and HFQT oscillations. Horizontal bars: Mean period during which dofetilide induced ventricular arrhythmias were observed. Green horizontal arrows and dotted lines: Period during which dofetilide induced ventricular arrhythmias were suppressed in the presence of atropine or hexamethonium. Vehicle: Red filled circles, drug alone: Blue filled circles, drug combined with atropine or hexamethonium: Blue empty circles. Data are presented as mean values ± SEM ($n = 6$, $^*P \leq 0.05$, when compared with vehicle)

effects. This work confirms the presence of haemodynamic effects with a majority of torsadogenic drugs directly visible on DAP and SAP. Two drugs only, quinidine and moxifloxacin, only caused QTc prolongation and had no haemodynamic and autonomic effects. It can be assumed that these two drugs act as pure hERG blockers devoid of haemodynamic side effects. Importantly, the QTc prolongation was lower in this first subgroup than that of the second subgroup represented by dofetilide and ibutilide. In this second group, the HFAM model supports the onset of a parasympathetic reflex associated with an increase in HFHR oscillations which in turn leads to an increase in HFQT oscillations. The hypothesis of a parasympathetic baroreflex activation is consistent with positive inotropic properties described for dofetilide and other hERG blocking drugs such as **E4031** and almokalant. Indeed, these hERG blockers have shown a positive inotropic effect on isolated cardiac tissues which correlates with their effects on VR and prolonged opening of voltage-gated calcium channels (Abrahamsson et al., 1993; Tande et al., 1990). Consistently, the positive inotropic effect is expected to increase stroke volume and cardiac output leading to an increase in BP. In turn, the baroreflex should be activated to counterbalance the increase in cardiac output mainly by slowing the HR. In fact, detailed examination of HFHR oscillations shows that the beat-to-beat HF slows down significantly, but over 1 to 2 beats only. At the peak of this transient bradycardia, the BP continues to fall. In the case of dofetilide, ibutilide, and astemizole, this decrease is enhanced by larger pauses and is visible at the peak of the HR oscillations on the minimum DAP value reached during the oscillations. This is the only directly visible indication of an effect of these molecules on BP. Apart from this effect, the mean DAP and SAP remained unchanged in the presence of this unusual mode of reflex compensation. This transient acceleration phase during the HF oscillations is thus responsible for the paradoxical increase in mean HR associated with these molecules. In contrast to the previous subgroup, the effects of the molecules in the third subgroup are not compensated by the reflex mechanisms involved. Indeed, these molecules all caused increases in MAP, DAP and SAP. We failed to find reports of specific haemodynamic mechanisms supporting these findings in the literature for pimozide, haloperidol and sertindole. Alternatively, positive inotropic effects related to stimulation of **5-HT$_4$ receptors** were reported for cisapride (Chai et al., 2012). This molecule supports the hypothesis of a positive inotropic mechanism causing a baroreflex parasympathetic activation responsible for the enhancement of HFHR oscillations. These torsadogenic hERG blockers induced a sympatho-vagal

coactivation. This specific autonomic mode was found to be largely predominant during the 24-h post-dose period for most of these molecules as well as for the molecules in the fourth subgroup of torsadogenic drugs. In contrast, the main feature of molecules of this last group was a mild decrease in SAP. Importantly, all torsadogenic hERG blockers of this last subgroup share a common off target, that is, **α_1 adrenoceptor** blocking properties: chlorpromazine (Hals et al., 1986), droperidol (Muldoon et al., 1977), risperidone (Nourian et al., 2008), thioridazine (Sleight et al., 1993). This property is consistent with the lowering of SAP. In addition, prazosin, an $\alpha_{1A/1B/1D}$-adrenoceptor inverse agonist (Alexander, Christopoulos, et al., 2021), was found to cause the same haemodynamic pattern as these drugs characterized by a mild decrease in SAP, a sympatho-vagal coactivation, and an enhancement of HFQT oscillations (available in raw data only). This phenomenon of sympatho-vagal coactivation has been directly recorded in dogs from left thoracic vagal nerve activity (NVA) and sympathetic left stellate ganglion nerve activity (SGNA). This specific autonomic pattern has previously been shown to precede episodes of orthostatic hypotension due to postural changes (Hellyer et al., 2014). According to these authors, the primary phenomenon leading to sympatho-vagal coactivation is SGNA while VNA is the secondary phenomenon that counterbalances the SGNA for BP and HR correction. In the case of α_1 adrenoceptors blocking drugs, the sequence of events could be the same, that is, first a sympathetic activation due to peripheral arterial vasodilation causing in turn a baroreflex sympathetic activation, then followed by a vagal coactivation to counterbalance the sympathetic activation for BP and HR control. The sympatho-vagal coactivation phenomenon observed with drugs causing increases in BP could also simply be due to a phenomenon of HR and BP correction but following a reverse sequence of events, first vagal activation in response to the increase in BP followed by transient sympathetic activation to counterbalance the vagal activation. These situations of sympatho-vagal coactivation have been artificially reproduced in anaesthetised dogs. Indeed, stimulation of the left stellate ganglion induces sympathetic activation of the left ventricle which in turn causes baroreflex vagal activation in response to increased inotropism. This situation of "forced" sympatho-vagal coactivation has been shown to trigger Torsades de Pointes in dogs when associated with prolongation of ventricular repolarization induced by a **I_{Ks} channel** blocker (Ter bekke et al., 2019). In Long QT1 (**KCNV1 channel** mutation) and long QT2 (hERG channel mutation) syndromes (LQTS), TdP is mainly triggered by sympathetic activation achieved in various situations such as physical exercise, swimming, fear reaction or arousal events during sleep (Kim et al., 2010; Schwartz et al., 2001). All these conditions could be associated with sympatho-vagal coactivation and/or reflect sudden and transient sequences of sympathetic activation. Syncope due to a hypotension episode is also expected to cause sudden sympathetic activation to restore BP. Syncopal events are associated with an increased risk of sudden cardiac death in LQTS (Jons et al., 2010; Moss et al., 1991). Orthostatic hypotension and associated vaso-vagal syncope constitute one of the most frequent adverse events induced by α_1 adrenoceptor blocking drugs (Carruthers, 1994). Overall, the proposed sympatho-vagal coactivation reflex mechanisms offer robust rational support to current therapeutic strategies applied in LQT syndromes based on β-blocker therapy or sympathetic left stellate ganglia ablation for prevention of the risk of sudden cardiac death (Priori et al., 2015).

The haemodynamic side effects of torsadogenic drugs could also have a strong impact on the assessment of the risk of QTc prolongation as they can compromise its evaluation. Indeed, this component is responsible for the phenomenon of concealed QTc prolongation at early doses for several torsadogenic hERG blockers tested in this work. It was initially reported for a high dose of thioridazine (Champéroux et al., 2010). Concealed QTc prolongation is due to sympathetic activation during sympatho-vagal coactivation as this phenomenon has been observed with most drugs causing sympatho-vagal coactivation and has been revealed under β-adrenoceptor blockade by atenolol. This phenomenon probably involves the recruitment of the I_{Ks} repolarisation reserve by β-adrenoceptors mediated sympathetic activation (Volders et al., 2003). For small molecules, the phenomenon of concealed QTc prolongation may likely contribute to the poor sensitivity in the 1×–10× exposure multiple ranges of the current preclinical strategy based on risk assessment of QT prolongation and hERG blockade (Park et al., 2018). Similar difficulties are met in the clinic to identify at risk patients affected by Long QT Syndromes (LQTS). Indeed, 25% approximately of genotyped LQTS patients presented concealed QT prolongation without correlated reduction of TdP risk (Goldenberg et al., 2011). The absence or very low incidence of ventricular arrhythmias and the phenomenon of concealed QTc prolongation in healthy dogs reported in this work for torsadogenic hERG blockers increase the difficulties of detecting new drug candidates at risk in preclinical studies. Similarly, none of them induced Torsades de Pointes, per se. Unlike healthy cynomolgus monkeys (Champéroux et al., 2015), dofetilide does not produce TdP in healthy beagle dogs and requires ventricular remodelling such as chronic atrioventricular block as a prerequisite for TdP induction (Dunnink et al., 2012). In fact, TdP is considered as reflecting propagation of unstable re-entrant "scrolls" across the ventricular wall maintained by trains of early afterdepolarizations (EADs) or by the heterogeneity of cardiac action potentials in the context of prolonged ventricular repolarisation (Roden, 2016). This abnormal propagation of ventricular action potentials is preceded and triggered by a ventricular arrhythmic event, possibly due to early afterdepolarization at cellular levels or by a re-entry phenomenon due to heterogeneity of cardiac action potentials duration across the myocardium or in the Purkinje fibres network in the case of bundle branch blocks. Heterogeneity of cardiac action potentials duration and beat-to-beat variability of repolarisation are largely enhanced by rate dependent mechanisms related to use or reverse-use dependency properties of voltage dependent ion channels (Wu et al., 2011). In vivo, HF parasympathetic oscillations are the main source of rate dependent heterogeneity of ventricular repolarisation. We showed that blocking these HF oscillations by atropine was sufficient to prevent dofetilide induced ventricular arrhythmias. In addition, we have highlighted that reappearance of ventricular arrhythmias coincided with the return to enhanced HF vagal oscillations and not with QT prolongation. Accordingly, these results and the

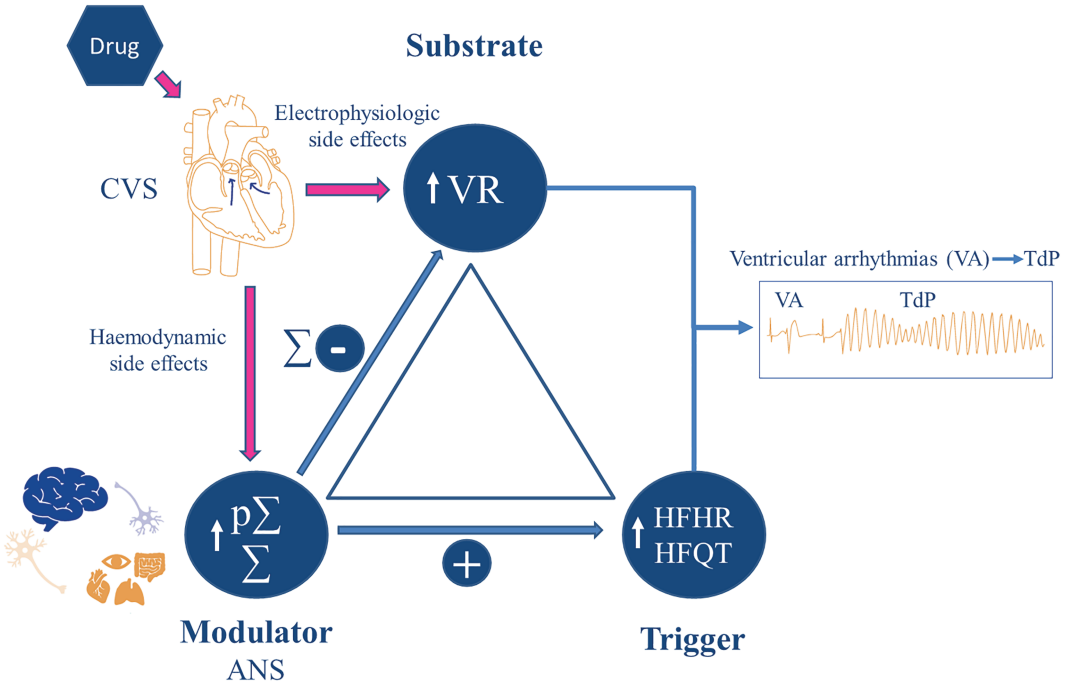

FIGURE 8 Update of the Coumel's triangle when applied to torsadogenic hERG blocking drugs. According the Coumel's triangle concept, ventricular arrhythmias and related TdP induced by torsadogenic hERG blocking drugs could require: (1) a substrate, the ventricular repolarisation (VR) lengthening due to electrophysiologic side effects; (2) a modulator, the autonomic nervous system (ANS), which is activated by haemodynamic side effects on the cardiovascular system (CVS). When present, the sympathetic component of HF oscillations can shorten the VR and conceals the QTc prolongation. (3) A trigger, the enhanced HFHR and HFQT oscillations themselves and their sympathetic component when coactivated, which increase the probability of rate-dependent ventricular arrhythmias under conditions of VR prolongation. Σ: Sympathetic nervous system, pΣ: Parasympathetic nervous system

enhancement of HFQT oscillations observed with almost all torsadogenic hERG blockers at early doses strongly support that their haemodynamic component may be a specific contributor to their in vivo arrhythmic profile. This specific contribution could have important implications for the clinic. Indeed, some discordances exist between pharmacovigilance alert thresholds and arrhythmic risk classification systems by CredibleMeds and CiPA (Davies et al., 2020). This haemodynamic component could open new avenues for improving patient stratification and drug risk assessment in prescribing algorithms for precision medicine.

In conclusion, these haemodynamic side effects may constitute a second component of their arrhythmic profile acting as a trigger alongside their intrinsic arrhythmogenic electrophysiological properties. This work establishes for the first-time such a relationship between torsadogenic drug induced haemodynamic effects leading to sympathetic compensatory reflexes and arrhythmic electrophysiologic mechanisms related to VR prolongation. The mechanisms of these haemodynamic side effects are multiple and all lead to an enhancement of vagal or sympatho-vagal HR oscillations associated with a paradoxical increase in mean HR. This relationship fits very well with the concept of the Coumel's triangle (Coumel, 1993) and allow us to refine and update dramatically (Figure 8) the model proposed earlier (Champéroux et al., 2015). According to this concept, TdP triggering could involve (1) a modulator: the autonomic nervous system: its contribution is characterized by an enhancement of HF oscillations related to parasympathetic or sympatho-vagal coactivation in response to haemodynamic side effects induced by vaso-active or inotropic torsadogenic drugs and/or due to VR prolongation itself for torsadogenic drugs acting selectively on VR. When present, the sympathetic component of HF oscillations can shorten the VR and conceals the QTc prolongation. (2) A substrate: the lengthening in VR due to intrinsic electrophysiological side effects in case of torsadogenic drugs which should not be limited to hERG channel blockade alone; and (3) a trigger: the enhanced HFHR and HFQT oscillations themselves and the sympathetic system which increase the probability of rate dependent arrhythmias under conditions of VR prolongation (Shimizu & Antzelevitch, 1999) during acceleration phases of HF cycles or following large RR pause, reminding that the majority of TdP in LQTS are pause dependent (Viskin et al., 2000).

AUTHOR CONTRIBUTIONS

Data analysis was performed by P.C. The manuscript was written by P.C., J.T. and J.Y.L. and critically evaluated by R.F. and S.R. Principal components analysis and hierarchical clustering analysis were performed by T.B.

CONFLICT OF INTERESTS

None declared.

DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the BJP guidelines for Design and Analysis, and Animal Experimentation, and as recommended by funding agencies, publishers and other organizations engaged with supporting research.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available at Figshare: doi: 10.6084/m9.figshare.19322975.

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REFERENCES

Abrahamsson, C., Duker, G., Lundberg, C., & Carlsson, L. (1993). Electrophysiological and inotropic effects of H 234/09 (almokalant) in vitro: A comparison with two other novel IK blocking drugs, UK-68,798 (dofetilide) and E-4031. *Cardiovascular Research*, *27*, 861–867. https://doi.org/10.1093/cvr/27.5.861

Alexander, S. P., Christopoulos, A., Davenport, A. P., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Southan, C., Davies, J. A., Abbracchio, M. P., Alexander, W., al-Hosaini, K., Bäck, M., Barnes, N. M., Bathgate, R., … Ye, R. D. (2021). THE CONCISE GUIDE TO PHARMACOLOGY 2021/22: G protein-coupled receptors. *British Journal of Pharmacology*, *178*(Suppl 1), S27–S156. https://doi.org/10.1111/bph.15538

Alexander, S. P., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Southan, C., Buneman, O. P., Cidlowski, J. A., Christopoulos, A., Davenport, A. P., Fabbro, D., Spedding, M., Striessnig, J., Davies, J. A., Ahlers-Dannen, K. E., … Zolghadri, Y. (2021). THE CONCISE GUIDE TO PHARMACOLOGY 2021/22: Introduction and other protein targets. *British Journal of Pharmacology*, *178*(Suppl 1), S1–S26. https://doi.org/10.1111/bph.15537

Alexander, S. P., Mathie, A., Peters, J. A., Veale, E. L., Striessnig, J., Kelly, E., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Southan, C., Davies, J. A., Aldrich, R. W., Attali, B., Baggetta, A. M., Becirovic, E., Biel, M., Bill, R. M., Catterall, W. A., … Zhu, M. (2021). THE CONCISE GUIDE TO PHARMACOLOGY 2021/22: Ion channels. *British Journal of Pharmacology*, *178*(Suppl 1), S157–S245. https://doi.org/10.1111/bph.15539

Carruthers, S. G. (1994). Adverse effects of alpha 1-adrenergic blocking drugs. *Drug Safety*, *11*(1), 12–20. https://doi.org/10.2165/00002018-199411010-00003

Chai, W., Chan, K. Y., de Vries, R., van den Bogeardt, A. J., de Maeyer, J. H., Schuurkes, J. A., Villalón, C. M., Saxena, P. R., Danser, A. H. J., & MaassenVanDenBrink, A. (2012). Inotropic effects of prokinetic agents with 5-HT(4) receptor agonist actions on human isolated myocardial trabeculae. *Life Sciences*, *90*(13–14), 538–544. https://doi.org/10.1016/j.lfs.2012.01.009

Champéroux, P., Fesler, P., Judé, S., Richard, S., Le Guennec, J. Y., & Thireau, J. (2018). High-frequency autonomic modulation: A new model for analysis of autonomic cardiac control. *British Journal of Pharmacology*, *175*, 3131–3143. https://doi.org/10.1111/bph.14354

Champéroux, P., Le Guennec, J. Y., Jude, S., Laigot, C., Maurin, A., Sola, M. L., Fowler, J. S. L., Richard, S., & Thireau, J. (2016). The high frequency relationship: Implications for torsadogenic hERG blockers. *British Journal of Pharmacology*, *173*, 601–612. https://doi.org/10.1111/bph.13391

Champéroux, P., Ouillé, A., Martel, E., Fowler, J. S., Maurin, A., Jude, S., Lala, P., Le Guennec, J. Y., & Richard, S. (2010). Interferences of the autonomic nervous system with drug induced QT prolongation: A point to consider in non-clinical safety studies. *Journal of Pharmacological and Toxicological Methods*, *61*(3), 251–263. https://doi.org/10.1016/j.vascn.2010.02.006

Champéroux, P., Thireau, J., Judé, S., Laigot-Barbé, C., Maurin, A., Sola, M. L., Fowler, J. S. L., Richard, S., & Le Guennec, J. Y. (2015). Short-term variability in QT interval and ventricular arrhythmias induced by dofetilide are dependent on high-frequency autonomic oscillations. *British Journal of Pharmacology*, *172*(11), 2878–2891. https://doi.org/10.1111/bph.13093

Coumel, P. (1993). Cardiac arrhythmias and the autonomic nervous system. *Journal of Cardiovascular Electrophysiology*, *4*, 338–355. https://doi.org/10.1111/j.1540-8167.1993.tb01235.x

Curtis, M. J., Hancox, J. C., Farkas, A., Wainwright, C. L., Stables, C. L., Saint, D. A., Clements-Jewery, H., Lambiase, P. D., Billman, G. E., Janse, M. J., Pugsley, M. K., Ng, G. A., Roden, D. M., Camm, A. J., & Walker, M. J. A. (2013). The Lambeth conventions (II): Guidelines for the study of animal and human ventricular and supraventricular arrhythmias. *Pharmacology & Therapeutics*, *139*(2), 213–248. https://doi.org/10.1016/j.pharmthera.2013.04.008

Dampney, R. A. (2016). Central neural control of the cardiovascular system: Current perspectives. *Advances in Physiology Education*, *40*(3), 283–296. https://doi.org/10.1152/advan.00027.2016

Davies, M. R., Martinec, M., Walls, R., Schwarz, R., Mirams, G. R., Wang, K., Steiner, G., Surinach, A., Flores, C., Lavé, T., Singer, T., & Polonchuk, L. (2020). Use of patient health records to quantify drug-related pro-arrhythmic risk. *Cell Reports Medicine*, *1*(5), 100076. https://doi.org/10.1016/j.xcrm.2020.100076

Dunnink, A., van Opstal, J. M., Oosterhoff, P., Winckels, S. K., Beekman, J. D., van der Nagel, R., Cora Verduyn, S., & Vos, M. A. (2012). Ventricular remodelling is a prerequisite for the induction of dofetilide-induced torsade de pointes arrhythmias in the anaesthetized, complete atrio-ventricular-block dog. *Europace*, *14*(3), 431–436. https://doi.org/10.1093/europace/eur311

Goldenberg, I., Horr, S., Moss, A. J., Lopes, C. M., Barsheshet, A., McNitt, S., Zareba, W., Andrews, M. L., Robinson, J. L., Locati, E. H., Ackerman, M. J., Benhorin, J., Kaufman, E. S., Napolitano, C., Platonov, P. G., Priori, S. G., Qi, M., Schwartz, P. J., Shimizu, W., … Zhang, L. (2011). Risk for life-threatening cardiac events in patients with genotype-confirmed long-QT syndrome and normal-range corrected QT intervals. *Journal of the American College of Cardiology*, *57*, 51–59. https://doi.org/10.1016/j.jacc.2010.07.038

Hals, P. A., Hall, H., & Dahl, S. G. (1986). Phenothiazine drug metabolites: Dopamine D2 receptor, alpha 1- and alpha 2-adrenoceptor binding. *European Journal of Pharmacology*, *125*(3), 373–381. https://doi.org/10.1016/0014-2999(86)90793-4

Hellyer, J., George Akingba, A., Rhee, K. S., Tan, A. Y., Lane, K. A., Shen, C., Patel, J., Fishbein, M. C., & Chen, P. S. (2014). Autonomic nerve activity and blood pressure in ambulatory dogs. *Heart Rhythm*, *11*(2), 307–313. https://doi.org/10.1016/j.hrthm.2013.11.022

Holzgrefe, H., Ferber, G., Champeroux, P., Gill, M., Honda, M., Greiter-Wilke, A., Baird, T., Meyer, O., & Saulnier, M. (2014). Preclinical QT safety assessment: Cross-species comparisons and human translation from an industry consortium. *Journal of Pharmacological and Toxicological Methods*, *69*(1), 61–101. https://doi.org/10.1016/j.vascn.2013.05.004

Holzgrefe, H. H., Cavero, I., Gleason, C. R., Warner, W. A., Buchanan, L. V., Gill, M. W., Burkett, D. E., & Durham, S. K. (2007). Novel probabilistic method for precisely correcting the QT interval for heart rate in telemetered dogs and cynomolgus monkeys. *Journal of Pharmacological and Toxicological Methods*, *55*(2), 159–175. https://doi.org/10.1016/j.vascn.2006.05.007

Jons, C., Moss, A. J., Goldenberg, I., Liu, J., McNitt, S., Zareba, W., Qi, M., & Robinson, J. L. (2010). Risk of fatal arrhythmic events in long QT

syndrome patients after syncope. *Journal of the American College of Cardiology*, *55*, 783–788. https://doi.org/10.1016/j.jacc.2009.11.042

Kannankeril, P., Roden, D. M., & Darbar, D. (2010). Drug-induced long QT syndrome. *Pharmacological Reviews*, *62*, 760–781. https://doi.org/10.1124/pr.110.003723

Kilkenny, C., Browne, W., Cuthill, I. C., Emerson, M., Altman, D. G., & NC3Rs Reporting Guidelines Working Group. (2010). Animal research: Reporting in vivo experiments: The ARRIVE guidelines. *British Journal of Pharmacology*, *160*, 1577–1579. https://doi.org/10.1111/j.1476-5381.2010.00872.x

Kim, J. A., Lopes, C. M., Moss, A. J., McNitt, S., Barsheshet, A., Robinson, J. L., Zareba, W., Ackerman, M. J., Kaufman, E. S., Towbin, J. A., Vincent, M., & Goldenberg, I. (2010). Trigger-specific risk factors and response to therapy in long QT syndrome type 2. *Heart Rhythm*, *7*(12), 1797–1805. https://doi.org/10.1016/j.hrthm.2010.09.011

Kramer, J., Obejero-Paz, C. A., Myatt, G., Kuryshev, Y. A., Bruening-Wright, A., Verducci, J. S., & Brown, A. M. (2013). MICE models: Superior to the HERG model in predicting torsade de pointes. *Scientific Reports*, *3*, 2100. https://doi.org/10.1038/srep02100

Lengyel, C., Varró, A., Tábori, K., Papp, J. G., & Baczkó, I. (2007). Combined pharmacological block of I (Kr) and I (Ks) increases short-term QT interval variability and provokes torsades de pointes. *British Journal of Pharmacology*, *151*, 941–951. https://doi.org/10.1038/sj.bjp.0707297

McGrath, J. C., & Lilley, E. (2015). Implementing guidelines on reporting research using animals (ARRIVE etc.): New requirements for publication in BJP. *British Journal of Pharmacology*, *172*, 3189–3193. https://doi.org/10.1111/bph.12955

Moss, A. J., Schwartz, P. J., Crampton, R. S., Tzivoni, D., Locati, E. H., MacCluer, J., Hall, W. J., Weitkamp, L., Vincent, G. M., & Garson, A. Jr. (1991). The long QT syndrome. Prospective longitudinal study of 328 families. *Circulation*, *84*(3), 1136–1144. https://doi.org/10.1161/01.cir.84.3.1136

Muldoon, S. M., Janssens, W. J., Verbeuren, T. J., & Vanhoutte, P. M. (1977). Alpha-adrenergic blocking properties of droperidol on isolated blood vessels of the dog. *British Journal of Anaesthesia*, *49*(3), 211–216. https://doi.org/10.1093/bja/49.3.211

Nourian, Z., Mulvany, M. J., Nielsen, K. B., Pickering, D. S., & Kristensen, T. (2008). The antagonistic effect of antipsychotic drugs on a HEK293 cell line stably expressing human alpha1A1-adrenoceptors. *European Journal of Pharmacology*, *596*, 32–40. https://doi.org/10.1016/j.ejphar.2008.08.014

Park, E., Gintant, G. A., Bi, D., Kozeli, D., Pettit, S. D., Pierson, J. B., Skinner, M., Willard, J., Wisialowski, T., Koerner, J., & Valentin, J. P. (2018). Can non-clinical repolarization assays predict the results of clinical thorough QT studies? Results from a research consortium. *British Journal of Pharmacology*, *175*(4), 606–617. https://doi.org/10.1111/bph.14101

Priori, S. G., Blomström-Lundqvist, C., Mazzanti, A., Blom, N., Borggrefe, M., Camm, J., Elliott, P. M., Fitzsimons, D., Hatala, R., Hindricks, G., Kirchhof, P., Kjeldsen, K., Kuck, K. H., Hernandez-Madrid, A., Nikolaou, N., Norekvål, T. M., Spaulding, C., van Veldhuisen, D., & ESC Scientific Document Group. (2015). ESC guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: The task force for the Management of Patients with ventricular arrhythmias and the prevention of sudden cardiac death of the European Society of Cardiology (ESC). *European Heart Journal*, *36*(41), 2793–2867. https://doi.org/10.1093/eurheartj/ehv316

Redfern, W. S., Carlsson, L., Davis, A. S., Lynch, W. G., MacKenzie, I., Palethorpe, S., Siegl, P. K., Strang, I., Sullivan, A. T., Wallis, R., Camm, A. J., & Hammond, T. G. (2003). Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: Evidence for a provisional safety margin in drug development. *Cardiovascular Research*, *58*(1), 32–45. https://doi.org/10.1016/s0008-6363(02)00846-5

Roden, D. M. (2016). Predicting drug-induced QT prolongation and torsades de pointes. *The Journal of Physiology*, *594*, 2459–2468. https://doi.org/10.1113/JP270526

Schwartz, P. J., Priori, S. G., Spazzolini, C., Moss, A. J., Vincent, G. M., Napolitano, C., Denjoy, I., Guicheney, P., Breithardt, G̈., Keating, M. T., Towbin, J. A., Beggs, A. H., Brink, P., Wilde, A. A. M., Toivonen, L., Zareba, W., Robinson, J. L., Timothy, K. W., Corfield, V., … Bloise, R. (2001). Genotype-phenotype correlation in the long-QT syndrome: Gene-specific triggers for life-threatening arrhythmias. *Circulation*, *103*, 89–95. https://doi.org/10.1161/01.cir.103.1.89

Shimizu, W., & Antzelevitch, C. (1999). Cellular basis for long QT, transmural dispersion of repolarization, and torsade de pointes in the long QT syndrome. *Journal of Electrocardiology*, *32*(Suppl), 177–184. https://doi.org/10.1016/s0022-0736(99)90077-8

Sleight, A. J., Koek, W., & Bigg, D. C. (1993). Binding of antipsychotic drugs at alpha 1A- and alpha 1B-adrenoceptors: Risperidone is selective for the alpha 1B-adrenoceptors. *European Journal of Pharmacology*, *238*(2-3), 407–410. https://doi.org/10.1016/0014-2999(93)90876-J

Tande, P. M., Bjørnstad, H., Yang, T., & Refsum, H. (1990). Rate-dependent class III antiarrhythmic action, negative chronotropy, and positive inotropy of a novel Ik blocking drug, UK-68,798: Potent in Guinea pig but no effect in rat myocardium. *Journal of Cardiovascular Pharmacology*, *16*, 401–410. https://doi.org/10.1097/00005344-199009000-00008

Ter Bekke, R. M. A., Moers, A. M. E., de Jong, M. M. J., Johnson, D. M., Schwartz, P. J., Vanoli, E., & Volders, P. G. A. (2019). Proarrhythmic proclivity of left-stellate ganglion stimulation in a canine model of drug-induced long-QT syndrome type 1. *International Journal of Cardiology*, *1*(286), 66–72. https://doi.org/10.1016/j.ijcard.2019.01.098

Thomsen, M. B., Verduyn, S. C., Stengl, M., Beekman, J. D., de Pater, G., van Opstal, J., Volders, P. G. A., & 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

Viskin, S., Fish, R., Zeltser, D., Belhassen, B., Heller, K., Brosh, D., Laniado, S., & Barron, H. V. (2000). Arrhythmias in the congenital long QTsyndrome: How often is torsade de pointes pause dependent? *Heart*, *83*, 661–666. https://doi.org/10.1136/heart.83.6.661

Volders, P. G. A., Stengl, M., van Opstal, J. M., Gerlach, U., Spätjens, R. L. H. M. G., Beekman, J. D. M., Sipido, K. R., & Vos, M. A. (2003). Probing the contribution of IKs to canine ventricular repolarization: Key role for beta-adrenergic receptor stimulation. *Circulation*, *107*, 2753–2760. https://doi.org/10.1161/01.CIR.0000068344.54010.B3

Wu, L., Ma, J., Li, H., Wang, C., Grandi, E., Zhang, P., Luo, A., Bers, D. M., Shryock, J. C., & Belardinelli, L. (2011). Late sodium current contributes to the reverse rate-dependent effect of IKr inhibition on ventricular repolarization. *Circulation*, *123*(16), 1713–1720. https://doi.org/10.1161/CIRCULATIONAHA.110.000661

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