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Hypothesis Article

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

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Short title: Hemodynamic side effects of torsadogenic hERG blocking drugs

Author contribution: Data analysis was performed by P.C. 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.

Abstract

Background and Purposes: Several 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) from telemetric data. The present work aims to characterise 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, pimozone, quinidine, risperidone, sotalol, sertindole, terfenadine, thioridazine) were assessed by telemetry in beagle dogs. Hemodynamic 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 with the High Frequency Autonomic Modulation (HFAM) model.

Key results: Except 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 hemodynamic side effects. These hemodynamic mechanisms were characterised by transient HR acceleration during HF oscillations. A phenomenon of concealed QT prolongation was unmasked for several torsadogenic hERG blockers under β -adrenoceptors blockade by atenolol. Resulting enhancement of HFQT oscillations was shown to contribute directly to triggering of dofetilide induced ventricular arrhythmias.

Conclusions and implications: This work supports for the first time a contribution of hemodynamic side properties to ventricular arrhythmias triggering by torsadogenic hERG blocking drugs. These hemodynamic side effects may constitute a second component of their arrhythmic profile acting as a trigger alongside their intrinsic arrhythmogenic electrophysiological properties.

Bullet point summary:

‘What is already known’:

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

‘What this study adds’:

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

‘Clinical significance’:

- Hemodynamic side effects of torsadogenic hERG-blocking drugs could trigger ventricular arrhythmias through associated autonomic reflexes.
- The hemodynamic component could open new avenues for drug risk stratification in precision medicine

Keywords: hemodynamic, hERG channel, HF oscillations, QTc prolongation, Torsades de pointes.

Abbreviations: BP: blood pressure; DAP: diastolic arterial pressure; HF: high frequency; HFAM: high frequency autonomic modulation; HFQT: high frequency QT oscillations; HR: heart rate; MAP: mean arterial pressure; SAP: systolic arterial pressure; VR: ventricular repolarisation

1. Introduction

Torsades de Pointes (TdP) are 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 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 channels 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 of the lengthening of VR itself, the beat-to-beat variability of VR (BVR) also plays a major role in TdPs (Thomsen et al., 2004; Lengyel et al., 2007). 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 QT interval. Interestingly, both magnitudes of HF oscillations of heart rate (HFHR) and of HF oscillations of QT interval (HFQT) were increased by several hERG blockers responsible for TdPs in dogs (Champ eroux et al., 2016) and their suppression by a ganglioplegic agent fully prevents dofetilide induced TdPs in cynomolgus monkeys (Champ eroux et al., 2015). We also showed that the increases in HFQT oscillations induced by hERG blockers may result from a sympathetic activation concomitant to HF parasympathetic oscillations (Champ eroux 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 characterise a particular state of the autonomic control induced by hERG blockers, named S2 oscillations which originate from and reflect a sympatho-vagal coactivation (Champ eroux 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 hemodynamic changes (Dampney, 2016). So, we focused on possible hemodynamic effects of hERG blocking drugs and their underlying consequences on HF oscillations using a large set of torsadogenic hERG blockers.

2. Methods

Torsadogenic drugs are defined as drugs with 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](#), [thioridazine](#).

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 n° CEEA-111) according to European directive 2010/63/UE on animal welfare. Reporting of experiments follows the ARRIVE guidelines (Kilkenny *et al.*, 2010; McGrath and 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-analyzed for this work.

All animal experiments were conducted in the following conditions described thereafter. Adult (3 males and 3 females per group for former studies or 6 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/kg, s.c.) and buprenorphine (0.01 mg/kg, s.c.). Anaesthesia was induced by thiopental (15–20 mg/kg, 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. 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 by groups of two to four animals at maximum with playing tools. Environmental parameters were recorded continuously and maintained within a fixed range, room temperature at 15–21°C at 45–65% relative humidity. The artificial day/night cycle was 12 h light and 12 h darkness with light on at 07:30 am. Drinking water was provided ad libitum. Solid diet (300 g/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 serve as their own control according to cross-over design with a washout period of 48 hours at minimum up to one 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, haloperidol), an ascending dose design was followed. In that case, two additional sessions, vehicle versus drug combined with atenolol (β_1 -adrenoceptors blocker) were performed on the same animals as far as possible. In that 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 ARTTM acquisition software (release 4.33, Data Sciences International, St Paul, MN, USA) or PonemahTM (release 6.33, Data Sciences International, St Paul, MN, USA).

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 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 (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) was 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: $QTc=10^{(\log QT-\beta*(\log RR-\log RR_{ref}))}$. This method requires many beats for each discrete QTc value ($n>250$ in dogs). All beats collected for 1-hour sequences were included in the calculation of QTc values. Mean values of all parameters were calculated within discrete 10-second sequences (binning) to match the HFAM model.

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-second sequences (Champ eroux et al, 2015, 2016, 2018). Examples of large HFHR and HFQT oscillations after pharmacological dosing with thioridazine and dofetilide are done in Figure 1.

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 (Champ  roux et al., 2018). This model is based on the principle that HFRR oscillations magnitude measured within discrete 10-second sequences are mainly dependent on the parasympathetic system while HFHR oscillations magnitude are dependent on both parasympathetic and sympathetic nervous systems. This model requires calculations of HFHR_{ref} and HFRR_{ref} values individually for each animal during a free-treatment 24-hour 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 (Champ  roux 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-second 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-second sequences where the parasympathetic system is predominant. They are characterised by well-shaped and stable beat-to-beat HR oscillations. The S2 oscillations meanwhile, correspond to

discrete 10-second sequences where both the parasympathetic system and the sympathetic system are co-activated. They were characterised by larger beat-to-beat HR oscillations (usually >80 bpm in dogs) than S1 oscillations and 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-second sequences where the magnitude of HR oscillations is blunted, i.e. 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 statistic distribution of these three simply defined states of the autonomic control calculated over short 10-second sequences. Accordingly, S1, S2 and S3 outcomes are expressed in proportion in % per hour. In 24-hour safety pharmacology studies, this time interval of one hour is the most appropriate to assess drug effect at steady state since calculated from numerous 10-second sequences (n=360/h). Only the HFAM index can be considered as directly related to the magnitude of parasympathetic or sympathetic activities (Champ eroux et al., 2018).

Arrhythmias and conduction troubles analysis

Arrhythmias and conduction troubles analysis were performed following the Lambeth conventions (Curtis et al., 2013). 10-seconds 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 (Holzgreffe 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-hour recordings from 655 control sessions. Qualification of arrhythmias was performed manually.

Statistical procedures

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 \pm 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.

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.

Data availability

Raw data i.e. 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-hour 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](https://doi.org/10.6084/m9.figshare.19322975)

Nomenclature of targets and ligands

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 et al., 2021a, 2021b & 2021c).

3. Results

Most of tested torsadogenic hERG blockers showed various hemodynamic 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 hours post-dosing except for cisapride, droperidol and terfenadine which showed delayed effects..

Electrophysiological, autonomic and hemodynamic effects

Two examples of large HFHR and HFQT oscillations are shown for dofetilide and thioridazine (Figure 1). Both drugs caused 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.

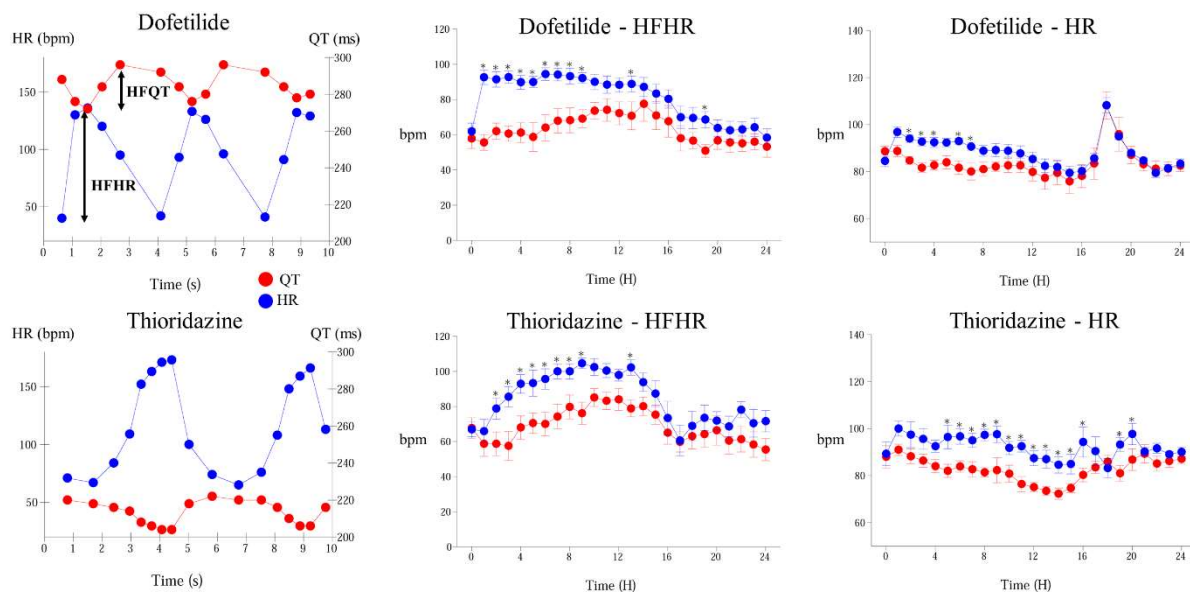


Figure 1. Left: Typical examples of HFHR oscillations (blue line) and related beat-to-beat HFQT oscillations (red line) induced by dofetilide (0.1 mg/kg, po) and thioridazine (1.5 mg/kg, po) in beagle dogs. HFQT oscillations are visible in the absence of QT prolongation for thioridazine and in the presence of QT prolongation for dofetilide. Middle and Right: Effects of dofetilide (1 mg/kg, po) and thioridazine (1.5 mg/kg, po) on HFHR and mean HR. Vehicle: red filled circles, drug alone: blue filled circles. Data are presented as mean values \pm SEM ($n=6$, *: $P \leq 0.05$, when compared to vehicle).

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. 4 molecules increased MAP, DAP and SAP, 3 others induced only mild decreases in SAP and 3 others caused only a decrease in minimum DAP (DAPmin) during HF oscillations (Figure 2).

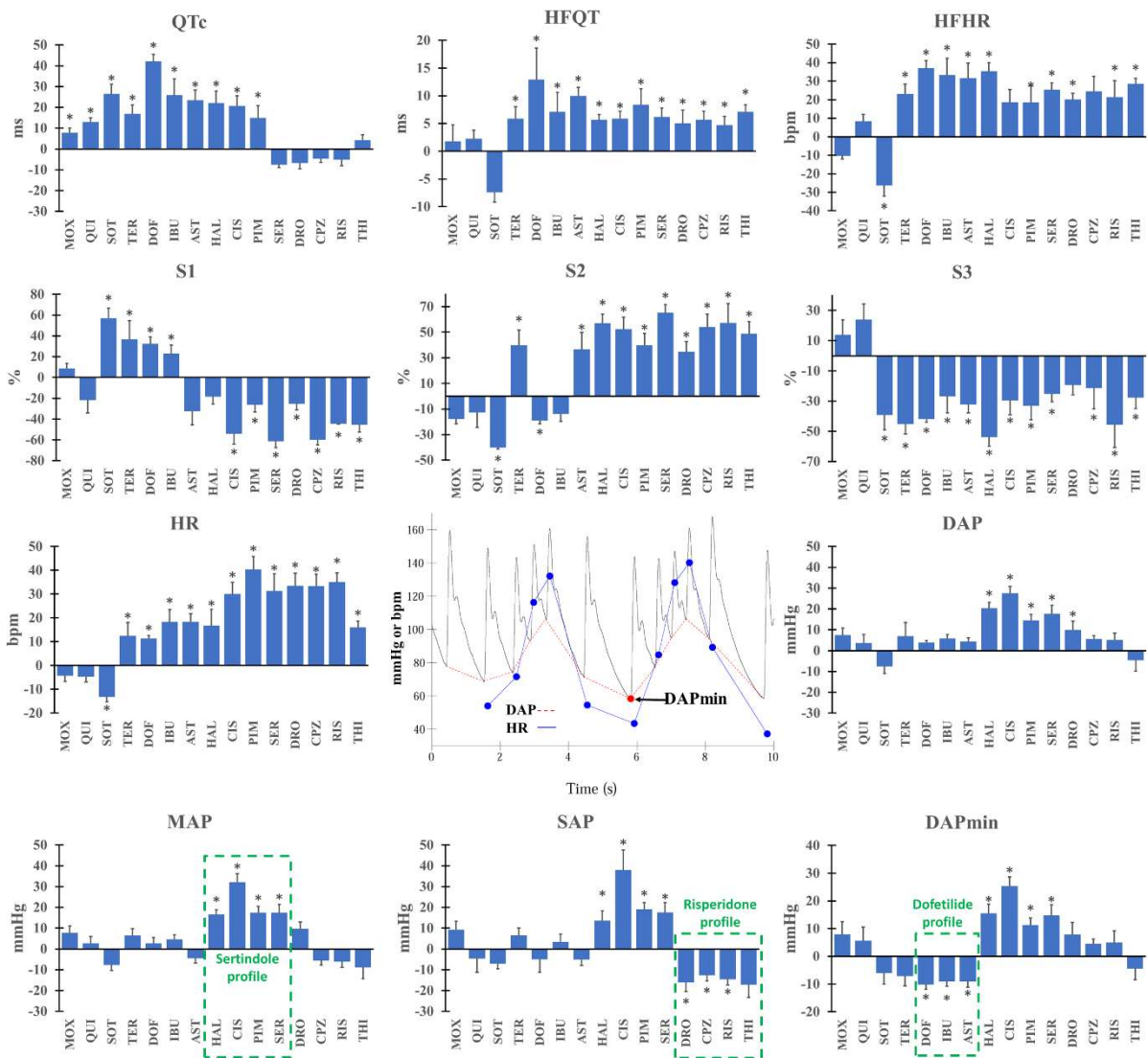


Figure 2. Effects of moxifloxacin (MOX, 10 mg/kg, po), quinidine (QUI, 10 mg/kg, po), sotalol (SOT, 10 mg/kg, po), dofetilide (DOF, 0.1 mg/kg, po), terfenadine (TER, 30 mg/kg, po), ibutilide (IBU, 1 mg/kg, iv), astemizole (AST, 1 mg/kg, iv), haloperidol (HAL, 3 mg/kg, po), cisapride (CIS, 6 mg/kg, po), pimoziide (PIM, 1 mg/kg, iv), sertindole (SER, 1 mg/kg, iv), droperidol (DRO, 3 mg/kg, iv), chlorpromazine (CPZ, 1 mg/kg, iv), risperidone (RIS, 1 mg/kg, iv) and thioridazine (THI, 1.5 mg/kg, po) 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

hemodynamic effects of dofetilide, sertindole and risperidone profiles are highlighted by dotted green boxes. Data are presented as mean values \pm SEM ($n=6$, *: $P \leq 0.05$, when compared to vehicle).

Both principal components analysis and hierarchical cluster analysis allow identification of 4 subgroups showing common haemodynamic, autonomic and electrophysiological features and profiles (Figure 3).

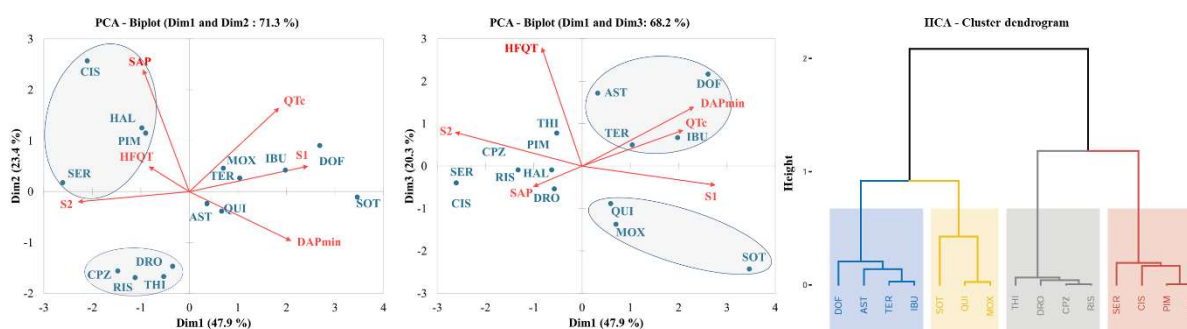


Figure 3. The 3 first components of the principal components analysis (PCA) explain more than 90% of the variance of the model reduced to 6 variables: SAP, decrease in DAPmin, QTc, S1, S2 and HFQT. Axes of the two first components (Dim1 and Dim2) allow to identify 2 first subgroups, SAP being the most contributing variable (58%) of the second component (Dim2). The first and the third components (Dim1 and Dim3) allow to identify 2 other subgroups, HFQT being the most contributing variable (69%) of the third component (Dim3). Hierarchical clustering analysis shows the same classification. AST (astemizole, 1 mg/kg, iv), CIS (cisapride, 6 mg/kg, po), chlorpromazine (CPZ, 1 mg/kg, iv), DOF (dofetilide, 0.1 mg/kg, po), DRO (droperidol, 3 mg/kg, iv), HAL (haloperidol, 3 mg/kg, po), IBU (ibutilide, 1 mg/kg, iv), MOX (moxifloxacin, 10 mg/kg, po), PIM (pimozide, 1 mg/kg, iv), QUI (quinidine, 10 mg/kg, po), RIS (risperidone, 1 mg/kg, iv), SER (sertindole, 1 mg/kg, iv), SOT (sotalol, 10 mg/kg, po), TER (terfenadine, 30 mg/kg, po) and THI (thioridazine, 1.5 mg/kg, po).

These profiles can be depicted by taking 4 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 hemodynamic 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 -adrenoceptors 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, po) 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 dosing. Transient decrease in DAP during HF oscillations (DAPmin) is observed at termination of the largest RR pause during HF oscillations (Figure 2). DAPmin values were lower with dofetilide. This was the only visible hemodynamic change observed with dofetilide (Figure 2). These low DAPmin levels were followed by transient sequences of HR acceleration over 2-3 beats likely to maintain unchanged both DAP and SAP values and compensate the hemodynamic 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 pimozone a profile characterised by an increase in MAP, DAP and SAP, predominant sympatho-vagal coactivation (S2) and enhancement of HFQT oscillations (Figure 2) following administration. These 4 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 characterised by a mild decrease in SAP, predominant sympatho-vagal coactivation (S2) and enhancement of HFQT oscillations (Figure 2). These 4 molecules also caused an increase in mean HR. None of these drugs caused directly visible QTc prolongation at early doses.

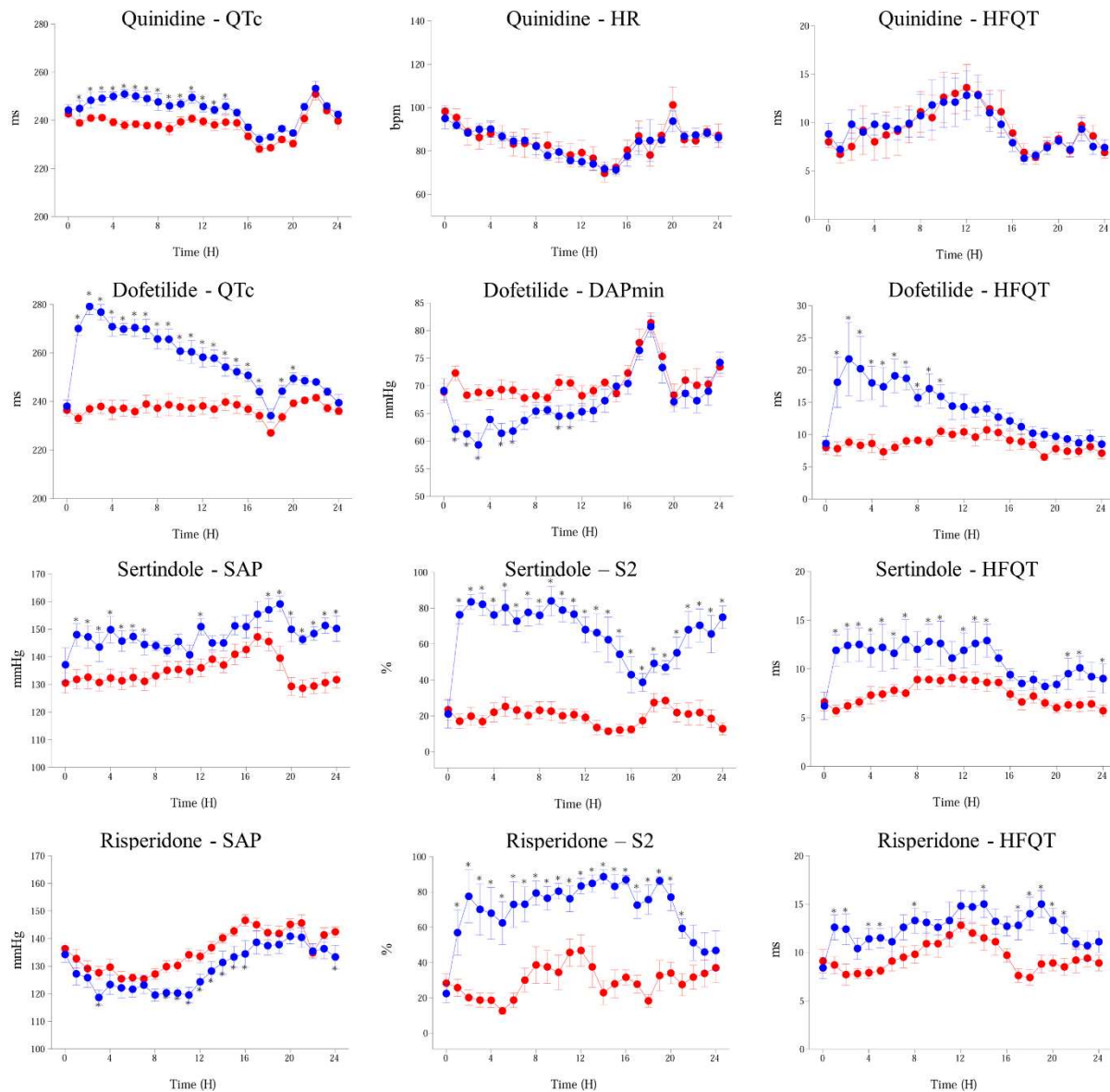


Figure 4. Four drugs were chosen to depict the various electrophysiologic, autonomic and hemodynamic profiles of the 15 torsadogenic hERG blockers. “Quinidine profile”: characterised by a mild QTc prolongation and the absence of any autonomic effects (mean HR and HFQT oscillations) due the absence of hemodynamic 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, po. Dofetilide: 0.1 mg/kg, po. Sertindole: 1 mg/kg, iv, Thioridazine: 1.5 mg/kg, po. Vehicle: red filled circles, drug alone: blue filled circles. Data are presented as mean values \pm SEM ($n=6$, *: $P \leq 0.05$, when compared to vehicle).

Concealed QTc prolongation

From signal analysis, we observed that 6 drugs induced concealed QTc prolongation. The QTc lengthening was unmasked when drugs were combined with atenolol, a β -adrenoceptors blocking drug (Figure 5). When given alone, atenolol is devoid of any electrophysiologic and hemodynamic effect (available in raw data only).

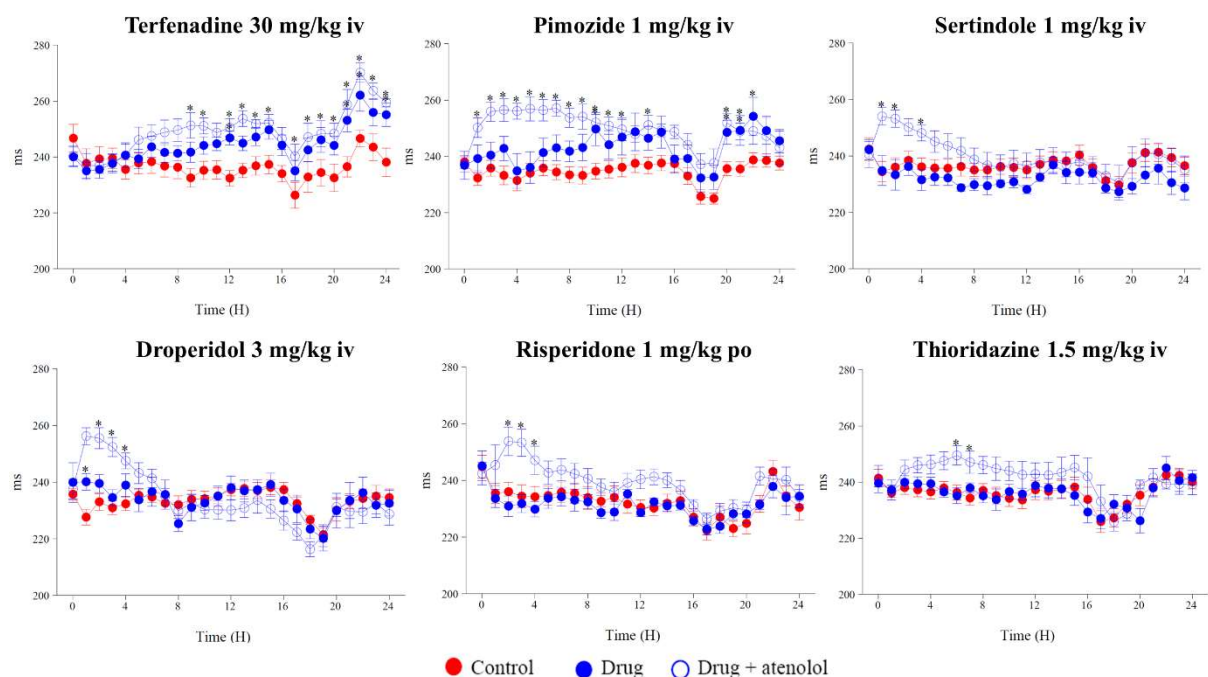


Figure 5. Examples of concealed QTc prolongation. In all these typical examples, drugs alone did not induce any visible QTc prolongation. QTc prolongation induced by these hERG channel blockers were unmasked in the presence of the β -blocker atenolol (1 mg>/kg/iv). Vehicle: red filled circles, drug

*alone: blue filled circles, drug combined with atenolol: blue empty circles. Data are presented as mean values \pm SEM (n=6, *: $P \leq 0.05$, when compared to vehicle).*

The arrhythmic component versus hemodynamic/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 characterised 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-hour 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.

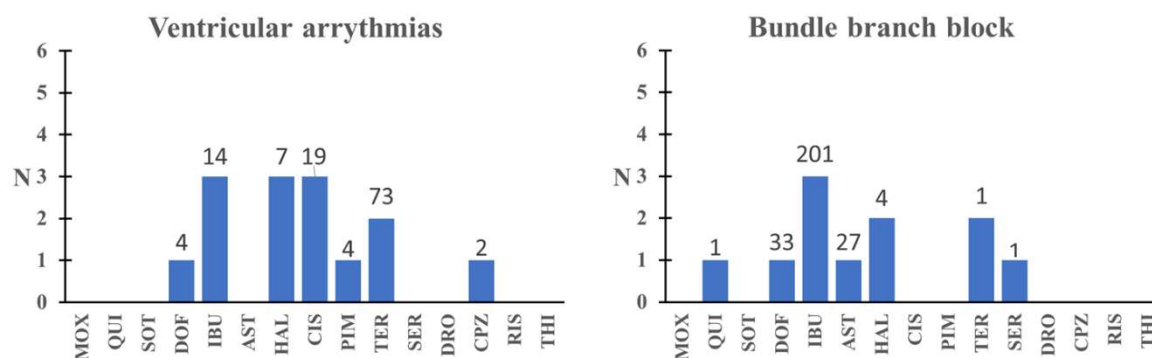


Figure 6. Summary bar graphs reporting the number of animals in each group showing ventricular arrhythmias (Left) and bundle branch blocks (Right) for the 15 torsadogenic hERG blocking drugs. Above each bar is mentioned the number of arrhythmic events or BBB detected over the 24-hour post dosing period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

The achievement of more reproducible ventricular arrhythmias in all animals with a higher incidence by the oral route was possible with dofetilide at the dose of 1 mg/kg 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 induced by dofetilide at a high dose (1 mg/kg, po) alone or under autonomic blockers (atropine and hexamethonium). It must be pointed that magnitude of QTc prolongation and HFHR/HFQT oscillations are similar at peak to that found at the lower dose of 0.1 mg/kg (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 were done 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 as the QT value and HF oscillations increase after dosing. Indeed, ventricular arrhythmias started on average at 1.4 ± 0.7 hours 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 hours post dosing while the onset of ventricular arrhythmias was delayed to 9 ± 1.1 hours (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 hours under hexamethonium, i.e. for the duration of the suppression of HF oscillations.

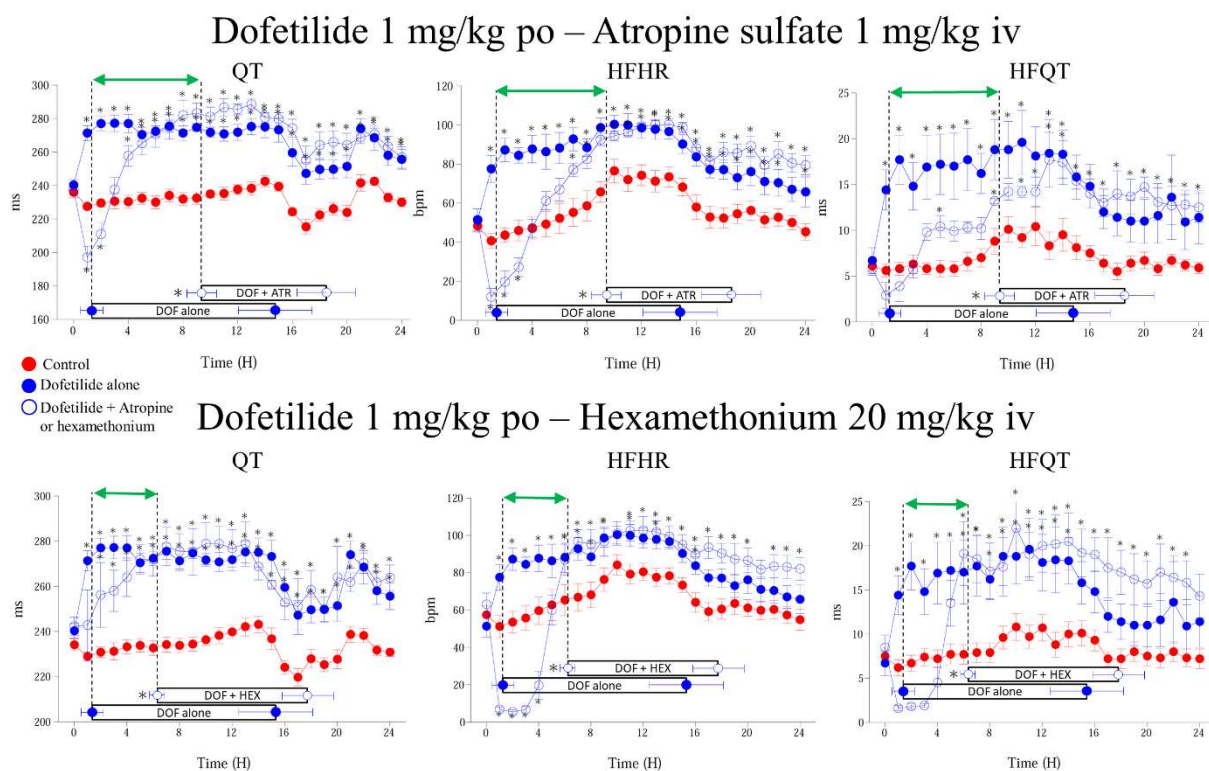


Figure 7. Upper panel: Effect of dofetilide (1 mg/kg, po) alone or in combination with atropine sulphate (1 mg/kg, iv) on QT interval, HFHR oscillations and HFQT oscillations. Lower panel: Effect of dofetilide (1 mg/kg, po) alone or in combination with hexamethonium (20 mg/kg, iv) on QT interval,

*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 \pm SEM (n=6, *: $P \leq 0.05$, when compared to vehicle).*

Discussion

This work was performed on a set of 15 hERG blocking torsadogenic drugs including 9 of the 10 most potent hERG blockers of the Kramer list (Kramer et al., 2013). This study aimed to characterise the underlying mechanisms responsible for sympatho-vagal coactivation and enhancement of HFHR and HFQT oscillations reported for several torsadogenic hERG blockers (Champ eroux 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 hemodynamic 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 hemodynamic and autonomic effects. It can be assumed that these two drugs act as pure hERG blockers devoid of hemodynamic 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 (Tande et al., 1990; Abrahamsson et al., 1993). Consistently, 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 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 hemodynamic mechanisms supporting these findings in the literature for pimozone, haloperidol and sertindole. Alternatively, positive inotropic effects related to stimulation of 5-HT₄ receptors were reported with 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-hour 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, i.e. α_1 adrenoceptors 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 in SAP. In addition, prazosin, an $\alpha_{1A/1B/1D}$ -adrenoceptors inverse agonist (Alexander et al., 2019a), was found to cause the same hemodynamic pattern as these drugs characterised 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, i.e. 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 IKs channel blocker (Ter bekke et al., 2019). In Long QT1 (KCNV1 channel mutation) and QT2 (hERG channel mutation) syndromes (LQTS), TdPs are mainly triggered by sympathetic activation achieved in various situations such as physical exercise, swimming, fear reaction or arousal events during sleep (Schwartz et al., 2001, Kim et al., 2010). All these conditions could be associated with sympatho-vagal coactivation and/or reflect sudden and transient sequences of sympathetic activation. Syncope due to 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 (Moss et al., 1991; Jons et al., 2010).

Orthostatic hypotension and associated vaso-vagal syncope constitute one of the most frequent adverse events induced by α_1 adrenoceptors 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 -blockers 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 (Champ eroux 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 -adrenoreceptor blockade by atenolol. This phenomenon probably involves the recruitment of the IKs 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 1x-10x 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 (Champ eroux 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 are 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 dependant 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 enhancement of HFQT oscillations observed with almost all torsadogenic hERG blockers at early doses strongly support that their hemodynamic 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 hemodynamic component could open new avenues for improving patient stratification and drug risk assessment in prescribing algorithms for precision medicine.

In conclusion, these hemodynamic 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 hemodynamic effects leading to sympathetic compensatory reflexes and arrhythmic electrophysiologic mechanisms related to VR prolongation. The mechanisms of these hemodynamic 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 characterised by an enhancement of HF oscillations related to parasympathetic or sympatho-vagal coactivation in response to hemodynamic 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, 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).

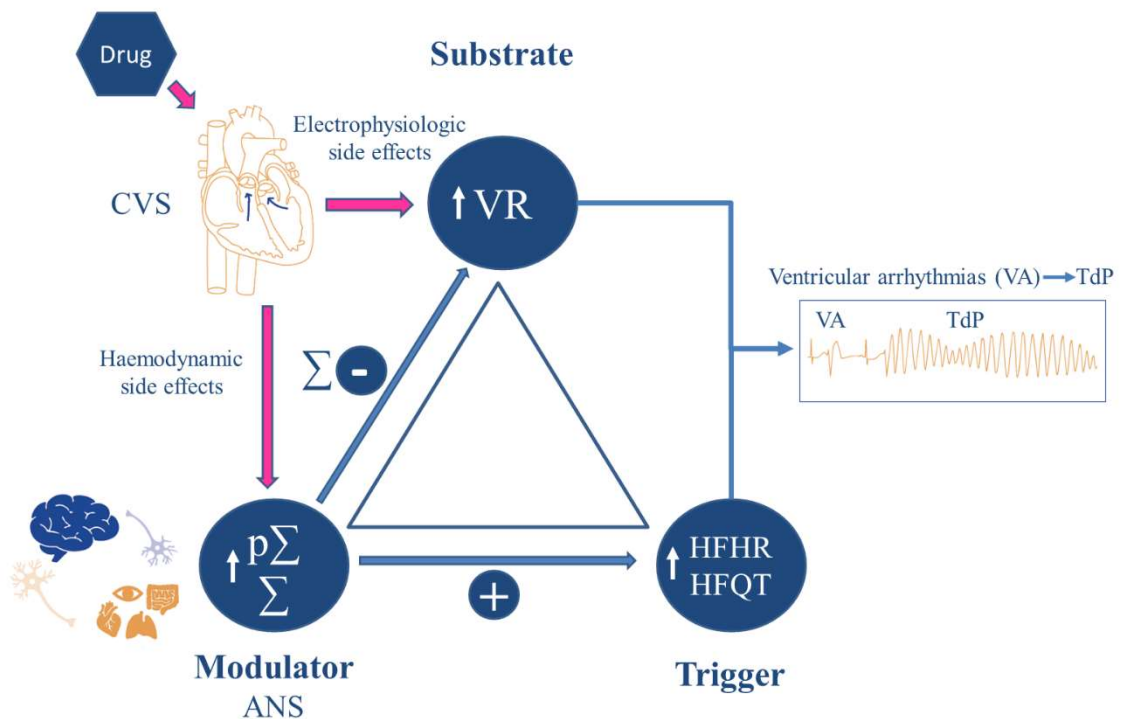


Figure 8. Update of the Coumel's triangle when applied to torsadogenic hERG blocking drugs. According the Coumel's triangle concept, ventricular arrhythmias and related TdP induced by torsadogenic hERG blocking drugs could require: 1/ A substrate, the ventricular repolarisation (VR) lengthening due to electrophysiologic side effects, 2/ A modulator, the autonomic nervous system (ANS), which is activated by hemodynamic 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, $p\Sigma$: parasympathetic nervous system.

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