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Review

# Use and abuse of QT and TRIaD in cardiac safety research: Importance of study design and conduct

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**Abstract**

Triangulation, reverse use dependence, instability and dispersion of ventricular repolarization (TRIaD), together with the cardiac wavelength ($\lambda$), are powerful proarrhythmic predictors. QT interval prolongation as such is not, as it provides false positives as well as false negatives. This has been established in various preclinical experiments on isolated tissues, perfused hearts and experimental animals, as well as in the clinic. Numerous risk factors including female gender, low serum potassium and magnesium, bradycardia and genetic predisposition, further promote arrhythmogenesis. Reliable quantitative preclinical cardiac safety studies using any experimental approach always require (1) rigorous adherence to Good Laboratory Practices (GLP), (2) collection of data only after complete drug equilibration has been established, (3) adequate resolution, (4) analysis based upon suitably powered statistical tests, (5) a sufficient number of experiments and (6) validated experimental protocols. Genesis of data not in accord with such standards and reported in an incorrect fashion confounds the significance of preclinical results for eventual clinical studies and elicits confusion regarding important drug effects. In this context, establishing a preclinical research consortium is suggested. This to assure: (1) the use of standardized experimental protocols and animal models; (2) data analysis with appropriately powered statistical tests; (3) correct testing and reporting and (4) elimination of some of the errors and abuses outlined in this paper.


*Keywords:* Drug safety; QT-liability; Repolarization disturbances; Good Laboratory Practice; Proarrhythmia

**Contents**



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## 1. Introduction

Drug-induced ventricular fibrillation and *torsade de pointes* form major problems in the development of safe new medications. Recent preclinical tests on isolated cardiac tissues and hearts (e.g., Hondeghem et al., 2001a,b; Lu et al., 2002; Hondeghem et al., 2003; Champeroux et al., 2005; Lawrence et al., 2005; Martin et al., 2006; Antzelevitch, 2007; Guo et al., 2007) and in experimental animals (e.g.:, De Clerck et al., 2002; Fossa et al., 2005; Schneider et al., 2005; Thomsen et al., 2006) identified various mechanisms for such drug-induced ventricular tachycardias. In the case of *torsade de pointes*, these mechanisms often extend beyond simple QT prolongation and they are modulated by numerous factors (e.g., female gender, low serum potassium and magnesium, bradycardia and/or genetic predisposition). Some of these factors (female gender, low potassium) have already been incorporated in sophisticated preclinical models (Hondeghem et al., 2001a; Eckardt et al., 2002). Furthermore, these achievements encourage research into supplemental techniques such as mapping of activation and three-dimensional cardiac electrical imaging to further enhance cardiac safety assessment (Walker et al., 2007; He et al., 2007).

To document these issues, the importance for arrhythmogenesis of differential effects on the characteristics of cardiac depolarization/repolarization will be evaluated, rather than focusing solely on the action potential duration. Next, requirements for their accurate use in drug safety assessment will be addressed.

## 2. QT prolongation and reverse use dependence

Life threatening ventricular tachycardias primarily result from disturbances of automaticity, conduction (Hoffman and Cranefield, 1964), and repolarization (Hondeghem et al., 2001a). It has been well established in the previous century that when $\lambda$ [cardiac wavelength = effective refractory period times conduction velocity] exceeds the length of the available path, then re-entry is blocked; while as $\lambda$ is shortened, re-entry is facilitated. Hence, it is not surprising that conduction velocity and effective refractory period form two main pillars in pro- and antiarrhythmic mechanisms. Based upon the modulated receptor theory (Hondeghem and Katzung, 1977), sodium channel blockers that also slow conduction of the sinus beat, were predicted to be less effective and perhaps unsafe (Hondeghem, 1987); the Cardiac Arrhythmia Suppression Trial (CAST) was terminated because these sodium channel blockers with slow kinetics increased mortality (Echt et al., 1991). Consequently development of drugs that caused prolongation of the effective refractory period using potassium channel blockers (i.e., class III antiarrhythmic agents) became popular. Unfortunately, effective refractory period prolongation by these drugs frequently declined as the heart rate increased: a phenomenon called *reverse* use dependence, which was similarly predicted to be less effective and perhaps unsafe under certain conditions (Hondeghem and Snyders, 1990). Indeed, the Survival With Oral D-Sotalol clinical trial (SWORD) was also terminated because such agents increased mortality (Waldo et al., 1996). In contrast, amiodarone prolongs the QT interval without inducing reverse use dependence (Sager et al., 1993), and slows conduction early in diastole without slowing conduction of the sinus beat [fast kinetics of interaction with the sodium channel (Mason et al., 1984)]. It is probably for these reasons that amiodarone is also a most effective antiarrhythmic agent.

*Torsade de pointes* is an arrhythmia (rarely) associated with the use of certain cardiovascular and non-cardiovascular drugs (www.torsades.org). *Torsade de pointes* was so frequently associated with QT interval prolongation that the latter became a surrogate for *torsade de pointes* (Roden, 2004). When drug-induced QT interval prolongation exceeds 8ms, it is considered a QT-liability (FDA Dockets Nos. 2004D-0377 and 2004D-0378) and results in a steep uphill battle for regulatory approval. Nevertheless, there are many widespread agents that prolong the QT interval but are not torsadogenic (e.g., carvedilol, ebastine, loratadine, mefloquine, phenobarbital, ranolazine, salbutamol, tamoxifen, tolterodine…).

Clearly, neither conduction slowing (e.g., by block of sodium channels) nor QT interval prolongation (e.g., by block of potassium channels) can be generalized as good or bad.

## 3. Triangulation

The strong association between *torsade de pointes* and QT prolongation may not be causal, but neither is it unexpected. Normally, action potential duration is primarily the sum of the plateau and fast repolarization phase of the action potential (Fig. 1). Thus, action potential duration can be prolonged by *delaying* (prolongation of plateau) or *slowing* of fast repolarization (prolongation of the fast repolarization phase). The latter was described as triangulation of the action potential (Hondeghem et al., 2001a). Triangulation results from a reduction in outward repolarizing currents and/or an increase of depolarizing inward currents during fast repolarization (in monophasic action potential recordings, dispersion of repolarization may also contribute to triangulation). It is important to stress that

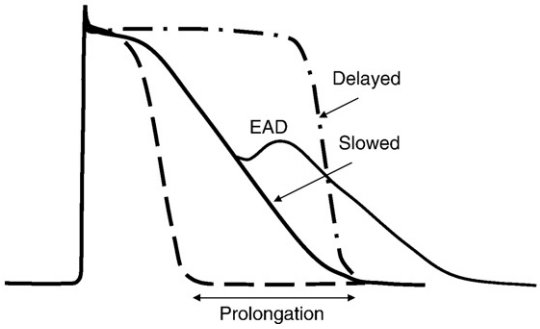


Fig. 1. An action potential (dashed line) can be prolonged to the same length by delaying prolongation (dot–dashed line) and/or slowing of repolarization (solid line). Slowed repolarization gives the action potential a more triangular appearance: triangulation. When slowing of repolarization becomes too marked, depolarization may stall or even lead to early afterdepolarizations (EAD).

triangulation can occur with prolongation, no change, or even shortening of the action potential duration. As inward currents approach outward currents, repolarization stalls; when inward currents exceed outward currents, then depolarization can yield early afterdepolarizations (see EAD in Fig. 1). Several thousand experimental observations in isolated rabbit hearts demonstrated that triangulation is highly proarrhythmic (Hondeghem et al., 2001a) and was confirmed by numerous laboratories (Milberg et al., 2002; Lu et al., 2002; Viitasalo et al., 2005; Champeroux et al., 2005; Martin et al., 2006; Guo et al., 2007; Milberg et al., 2007).

Two of aforementioned studies highlight important aspects of triangulation. First, terodiline and tolterodine (anti-urinary incontinence agents), both block hERG (human ether-à-go-go) channels in sub-therapeutic concentrations (Fig. 2); however, one has been shown to be associated with *torsade de pointes* while the other has not; terodiline causes marked triangulation without prolonging the action potential duration; tolterodine can prolong the action potential duration but without triangulation. QT-liability predicts that tolterodine would be more proarrhythmic than terodiline; but it is terodiline that was removed from the market because of proarrhythmia, while tolterodine is well tolerated. Elegant voltage clamp experiments (Fig. 2) demonstrated that tolterodine altered the voltage-dependence of hERG channels in such a way that during repolarization the outward potassium current was less depressed or even enhanced (Martin et al., 2006). Thus, not considering block of ion channels, as a function of time- and voltage-dependence (Hondeghem and Katzung, 1977) can be highly misleading, i.e., block of hERG does not necessarily render a drug torsadogenic.

Second, when comparing a series of hERG blocking quinolones (Milberg et al., 2007), QT prolongation was consistently observed in isolated rabbit hearts. However, only hearts that exhibited triangulation and dispersion went on to develop *torsade de pointes*. The clinical counterpart is that *torsade de pointes* usually only develops in a small fraction of patients, and efforts to recognize vulnerable patients need to extend beyond QT prolongation (Shah and Hondeghem, 2005).

## 4. Instability

When a drug prolongs $\lambda$ beyond the available path then reentry becomes impossible (Hoffman and Cranefield, 1964). An exception to this rule develops when the action potential duration becomes unstable (Hondeghem et al., 2001a) and yields out-of-phase oscillations of the action potential duration (Qian et al., 2003), i.e., temporal dispersion of the action potential duration. Excitatory waves of depolarization can then travel through tissue with short action potential duration, and around tissue with long action potential duration. Due to the long–short oscillations of action potential duration, the excitatory wave twists through ever-changing paths: *torsade de pointes*. In the majority of cases (~ 85%) *torsade de pointes* eventually runs out of excitable tissue and becomes self-terminating. However, as $\lambda$ shortens (e.g., reverse use dependence, ischemia) instability of effective refractory period may escalate and result in wave fractionation and deterioration into ventricular fibrillation (~ 15%). The importance of instability of repolarization was recently also illustrated for atrial fibrillation (Gong et al., 2007).

## 5. Dispersion

Because ion channels are not uniformly distributed in the heart, drug-induced changes in repolarization may also vary and result in *spatial* dispersion of the action potential duration (Antzelevitch, 2007), but also in dispersion of triangulation, reverse use dependence, and instability. Because of incomplete synchronization between various areas, instability can yield out-of-phase oscillations (Qian et al., 2003) or *temporal* dispersion. Where long and short action potentials collide, gradients exceeding 100mV per mm can result in strong intercellular currents. These can cause depolarizations, including early

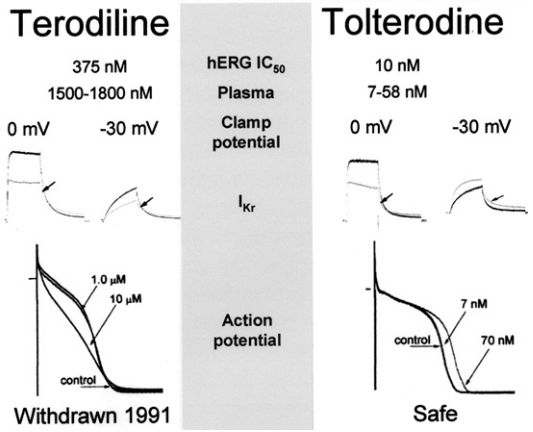


Fig. 2. Effects of terodiline and tolterodine upon $I_{Kr}$ and action potential. At therapeutic plasma concentrations hERG channels are blocked. While terodiline reduces $I_{Kr}$ at 0 mV (representative of the plateau potential) and −30 mV (during repolarization), tolterodine only reduces it at 0 mV, but increases $I_{Kr}$ at −30 mV. As a result, terodiline can triangulate the action potential, whereas tolterodine does not. While tolterodine can prolong the action potential, terodiline does not. Nevertheless, it is terodiline that was withdrawn from the market, while tolterodine is considered safe. Figure adapted with permission from Martin et al. (2006).

afterdepolarizations and oscillations, even in ordinary ventricular myocardium (Katzung et al., 1975).

It is to be expected that emerging techniques for multi-dimensional mapping of cardiac activation and action potential duration (Walker at al., 2007; He et al., 2007) will further document temporal and spatial inhomogeneities. This will probably improve the detection power of the present models.

## 6. TRIaD

Triangulation, reverse use dependence, instability and dispersion (TRIaD) form a primary proarrhythmic predictor (Hondeghem, 2006). TRIaD combined with prolongation of $\lambda$ (e.g., by QT prolongation) leads preferentially to *torsade de pointes*; TRIaD combined with shortening of $\lambda$, yields preferentially ventricular fibrillation. Thus, even in the presence of TRIaD, QT prolongation reduces proarrhythmia from ventricular fibrillation (usually lethal) to *torsade de pointes* (usually self-terminating). TRIaD has been extensively validated in blinded trials on isolated female rabbit hearts (Hondeghem and Hoffmann, 2003; Hondeghem et al., 2003; Valentin et al., 2004; Lawrence et al., 2005) and so far not a single agent is known to precipitate *torsade de pointes* without TRIaD. In contrast, *torsade de pointes* can frequently be observed without QT interval prolongation (Shah and Hondeghem, 2005).

Although the remainder of the article will focus on disturbances of repolarization, it cannot be emphasized enough that disturbances in conduction, effective refractory period, and automaticity are at least equally important components of drug safety evaluation.

## 7. Requirements for accurate use of TRIaD in safety assessment

In Table 1, requirements essential for the accurate use of TRIaD in safety assessments are listed; some examples of defaults, hampering quality research are given. Such defaults regarding context, experimental conditions and results should be banned, as they can seriously compromise the true significance of experiments for clinical settings.

### 7.1. Good Laboratory Practice (GLP)

Good Laboratory Practice (GLP) requires that solubility, stability, homogeneity and traceability of chemicals are fully characterized (OECD, 1997; Beernaert and Peeters, 2006). For definitive safety studies, it is advisable to archive a small aliquot of chemical/raw material so as to enable check on concentration and on stability of the test item in its solvent, should this appear useful.

Equally important, tissue bath concentration cannot be assumed to be identical to the nominal concentration, as the chemicals may adsorb to plastic/glass lines and vessels (Yahya et al., 1988; Palmgren et al., 2006). For example, when dofetilide is injected into the bubble trap, immediately above the heart, initial signs of TRIaD appear at concentrations ~ 3nM

Table 1
The first column refers to the subheadings in Section 7 while in the second column a brief example is given of the violation

| | |
|---|---|
| 7.1 | Good Laboratory Practice (GLP): …identify source, solubility, stability of chemicals & validate calculated results versus "wet lab" data with the original researchers… (Lu et al., 2006):<br>● No validation by original researcher of published results versus his "wet lab" data<br>● Discrepancies between published data and subsequent analysis by original researcher<br>● No identification of drug source or solvent, solubility/stability data in solvent; e.g., maximum solubility of grepafloxacin=66 μM (Bryskier, 1977), but studied at "300 μM"<br>● This non-identified "grepafloxacin" had no effects, but in identical conditions grepafloxacin became torsadogenic above 20 μM (Lawrence et al., 2006) |
| 7.2 | Equilibration: …study drug effects at steady state… (Lu et al., 2006):<br>● Equilibration periods (15 min) too short to reach steady state in the heart; e.g., erythromycin requires 45 min to approach full effect (Hondeghem et al., 2001b)<br>● Notifications by the original researcher on this were disregarded, so published effects are only an unknown fraction of true drug effects |
| 7.3 | Resolution: …sufficiently small concentration steps must be tested… (Lu et al., 2006):<br>● Ranking agents with $C_{max}$ of 3.4 to 6.3 μM, requires more resolution than 3- to 10-fold concentration steps |
| 7.4 | Statistics: …use appropriate statistical test and number of experiments… (Lu et al., 2006):<br>● 12 sparfloxacin experiments were done by the original researcher, but only 10 are reported of which only 6 were selected for analysis<br>● Figures in publication show early afterdepolarization in all 6 selected experiments, while the table reports 0/6<br>● Above 9 μM telithromycin, 3 out of 6 preparations terminated because of excessive proarrhythmia, but statistical analysis continues with an inappropriate $n=6$<br>● Used statistical test declares 3 instances not significant |
| 7.5 | Number of experiments: …must be sufficient to provide appropriate power…<br>● Statistics on 3 TRIaD experiments with azimilide and disopyramide are insufficient to classify as false negatives (Lawrence et al., 2006)<br>● Incorrect reporting: figures in publication show early afterdepolarizations in all 6 experiments, selected out of 10, while the table reports 0/6 (Lu et al., 2006) |
| 7.6 | Extrapolation of proarrhythmia to the clinic: …chemical with a 30–100 fold ratio of TRIaD to therapeutic range need large preclinical study samples… (Lu et al., 2006): ranking agents with such a similar liability requires more than $n=6$ TRIaD experiments |
| 7.7 | Gold standards: …for preclinical versus clinical ranking of liabilities, both rankings must be accurate… (Lu et al., 2006):<br>● The "gold standard" by which clinical effects are compared with TRIaD data on 4 antibiotics does not exist: "the available data is not quantitative and has too large error margins" (Dr. Woosley, personal communication)<br>● The preclinical data violate 5 of the above points, and thus misrepresent the expected clinical safety margin |
| 7.8 | Lack of validation: …for chemicals that prolong the plateau of the action potential, QT-liability may falsely incriminate a safe chemical… (Lawrence et al., 2006) prolongation of repolarization without TRIaD induction is used as an non-validated liability factor: "…torsade de pointes "may be less related to degree of QT prolongation than to drug effects on transmural dispersion or variability of repolarization" (Kannankeril and Roden, 2007)" |
| 7.9 | Safety margins: …no known clinical torsadogenic liability when threshold TRIaD exceeds upper therapeutic plasma concentration by at least 100… (Lawrence et al., 2006)<br>● Vardenafil is reported inappropriately as a "false positive" in TRIaD tests versus the clinic |

Table 1 (*continued*)

| | |
|---|---|
| | ● However, the ratio of the lowest TRIaD sign to target concentration PDE5 effects is 33,708 and well beyond the most conservative safety margin (Shah, 2005) |
| 7.10 | Completeness of lambda and TRIaD: …lambda and TRIaD evaluation is required to fully eliminate proarrhythmic potential… (Thomsen et al., 2006) |
| | ● Absence of instability only (beat-to-beat-variability of repolarization) in dogs used to declare moxifloxacin as clinically safe |
| | ● In vitro test detected TRIaD and clinical TdP was reported (Dale et al., 2007) |

(see Fig. 3). However, when perfused from a glass Erlenmeyer through a heat exchanger of 4 m polyethylene tubing, potency drops an order of magnitude (unpublished observation). Thus, for quantitative safety work it is highly desirable to collect samples at the entrance to the aorta and verify that the tissue gets exposed to the intended drug concentrations.

### 7.2. Equilibration

For quantitative safety analyses, it is a basic principle to obtain steady state drug effects. When evaluating drugs under preliminary 'screening' conditions, non-steady state detection of TRIaD may be useful for quick elimination of proarrhythmic agents. However, steady state must be achieved for safety studies, as otherwise drug safety will be overestimated (Steidl-Nichols et al., 2008).

In particular for experiments performed by external laboratories, it is imperative that test results are checked and validated for correctness by the original researcher versus his "wet lab data", before a publication of "work performed by others".

### 7.3. Resolution

When ranking drug safety for agents with similar therapeutic and toxic concentrations, then the concentration steps must be sufficiently small. Indeed, when therapeutic and proarrhythmic concentrations are separated many 100-fold, then correct ranking may not be very relevant. However, for smaller separations less correct ranking may make the difference between rare and extremely rare proarrhythmic (but potentially lethal) events difficult.

### 7.4. Statistics

Levels for statistical significance of TRIaD were defined as deviations exceeding the 97.5% confidence intervals from data derived from 142 drug free experiments (Hondeghem et al., 2003). An alternative and perhaps better way would be to determine the 97.5% confidence intervals of TRIaD resulting in

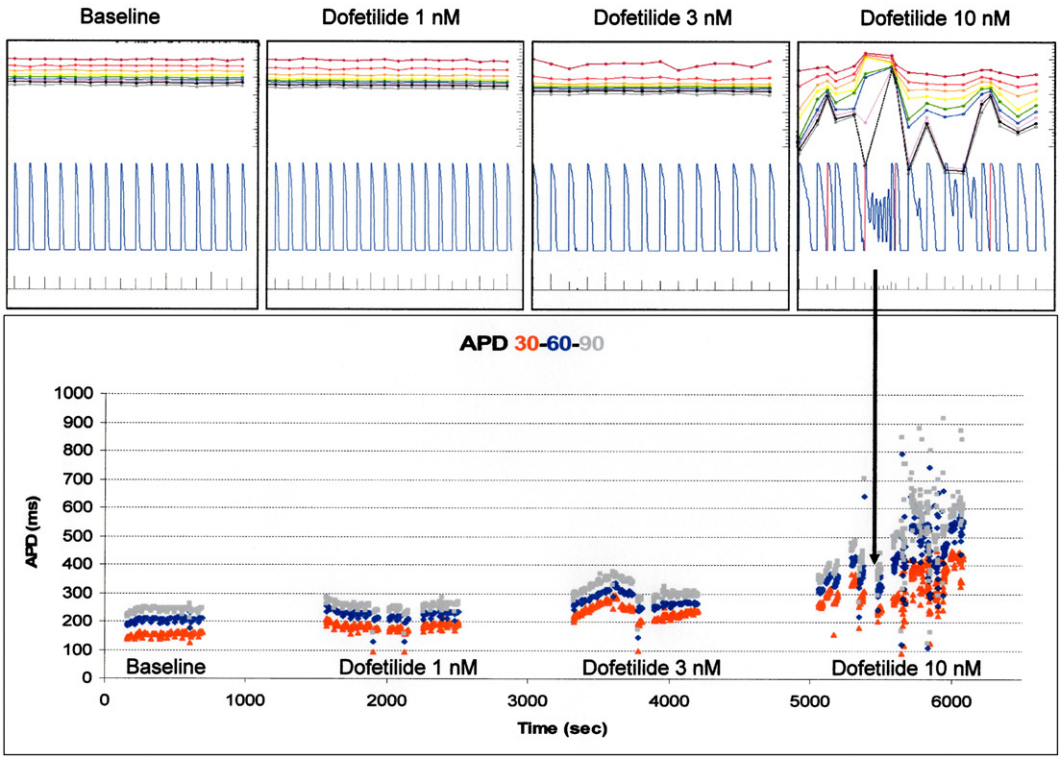


Fig. 3. Effects of dofetilide upon the cardiac monophasic action potential (1 Hz). In the top 4 panels (baseline, 1 nM, 3 nM and 10 nM) the action potential durations to 10 (brown), 20 (red), 30 (orange), 40 (yellow), 50 (green), 60 (blue), 70 (violet), 80 (dark grey) and 90% (light grey) repolarization are plotted for each action potential (blue traces). At 1 and 3 nM a small prolongation of the action potential duration developed and the first signs of instability became apparent (see undulations in action potential durations 10 and 20%). At 10 nM triangulation and instability became very marked and they resulted in early afterdepolarizations, non-stimulated ectopic beats (red upstrokes) and *torsade de pointes* (top right panel). In the bottom panel action potential durations at 30, 60 and 90% repolarization are shown. The arrow indicates where the first *torsade de pointes* was recorded.

drug-induced *torsade de pointes* or ventricular fibrillation. Indeed, should the control and proarrhythmic TRIaD confidence intervals overlap, false negatives for tested substance might result. Conversely, to the extent that the upper limits of the control values are smaller than proarrhythmic lower limits, false positives might also result. Unfortunately, the latter (potentially better) approach must await the availability of a large enough data base of drug-induced proarrhythmia before such safety limits can be validated: initial attempts indicate that these confidence intervals are a complex and non-linear function of $\lambda$ (Hondeghem, 2006). Until then, drug-induced TRIaD beyond the 97.5% drug free confidence intervals is abnormal, and may be proarrhythmic. So far, analysis based upon magnitude and frequency of such deviations has reliably recognized drugs that are proarrhythmic in patients without falsely incriminating safe agents (Hondeghem and Hoffmann, 2003; Hondeghem et al., 2003; Valentin et al., 2004). Although this may suggest that the overlap between the control and proarrhythmic TRIaD limits may neither be overlapping nor separated much, it will require experimental substantiation.

Unfortunately, the sensitivity of the validated tests against a sufficiently large series of controls is not always fully appreciated and standard statistical approaches (ANOVA, Mann–Whitney, Students *t*-test, Fisher's exact test, etc.) for data analysis appear inadequate when applied to TRIaD. A simple example can best illustrate this important point. When comparing a group ($n=6$) with no untoward events (e.g., drug free control) to a drug group ($n=6$; Fisher's test; $p<0.05$ for significance), then there must be at least 5 events in the treated group to attain significance. Thus, using this logic, a drug that precipitates *torsade de pointes* or ventricular fibrillation in 4 out of 6 experiments could then be considered safe (a pure fallacy). However, when appropriately using the Fisher's test against the baseline group of 142 observations with no instances of *torsade de pointes* or ventricular fibrillation, then 1 *torsade de pointes* or 1 ventricular fibrillation in 6 experiments does yield statistical significance.

### 7.5. Number of experiments

Number of experiments must also be sufficient to provide appropriate power to the safety evaluation. Important parameters include: level of risk that is acceptable, importance of the medical benefit and the incidence of the proarrhythmic promoter. This is a very complex issue that goes beyond the scope of the present review. While observing no TRIaD in 3 experiments may be important when evaluating continued drug development, in safety studies much larger numbers are required to adequately rule out a proarrhythmic potential.

### 7.6. Extrapolation of proarrhythmia to the clinic

Extrapolation of proarrhythmia to the clinic is reliable when the TRIaD signs are detected in virtually all experiments and occur close to the therapeutic concentration. As the proarrhythmic potential of a chemical declines, more experiments and/or higher concentrations are required before significant TRIaD levels become detected. In general, when significant TRIaD develops at less than 30-fold above therapeutic concentration, then a chemical becomes highly likely to generate detectable clinical proarrhythmia (Lawrence et al., 2006). So far, no drug that is devoid of significant TRIaD up to 100-fold therapeutic concentration is known to elicit detectable *torsade de pointes*. For the chemicals having a 30- to 100-fold ratio of TRIaD to therapeutic range, the clinical incidences become very low ranging from $\sim$1 per thousand to $\sim$1 per million. In such cases larger preclinical studies and more careful clinical monitoring are warranted.

### 7.7. Gold standards

When testing the accuracy of preclinical ranking against clinical ranking, it is essential that both rankings are accurate. In order to rank drugs that have very low reported incidences of *torsade de pointes*, (e.g., 0.1 to 15 per million) with (1) only a 90% chance to resolve a 0.1 per million difference over the above range, and (2) a 5% level of significance between only two drugs, a trial in 15,719,315,723 patients is required (http://stat.ubc.ca/~rollin/stats/ssize/b1.html). Today the world's population renders such a trial impossible. Dr. R. Woosley, who is responsible for the www.torsades.org website, pointed out that ranking such agents for their relative risk of inducing *torsade de pointes* is not possible: "the available data used for the decision criteria of detection and placement on the website list is at the moment not quantitative and has too large error margins" (personal communication). Correct ranking of such small differences would similarly require sufficiently large *n*-values in preclinical studies as well.

Furthermore, for a study to detect such small differences between drugs, all factors modulating proarrhythmia should be equally distributed between the study groups. These include but are not limited to (1) serum concentrations of potassium and magnesium, (2) heart rate, (3) autonomic tone (alpha, beta and parasympathetic as influenced by blood pressure, exercise, medications, hormones, food), (4) drug metabolism (including influences of co-medication) and metabolites, (5) age, (6) gender, (7) cardiac calcium overload as frequently associated with diseases (e.g., congestive heart failure), (8) genetic polymorphisms (especially with respect to ion channels and drug metabolism), (9) rhythm disturbances (e.g., atrial fibrillation and atrio-ventricular block), (10) disease (kidney, liver, heart, HIV infection). As a result very rare incidents of drug-induced proarrhythmia are impossible to accurately quantify, which renders development of a gold standard impossible.

### 7.8. Lack of validation

In addition to the validated proarrhythmic vectors (shortening of $\lambda$ and increased TRIaD), it is not uncommon to also include QT-liability or even substitute only the latter. In conditions where the chemicals studied prolong action potential duration by triangulation, this will further emphasize the weight of triangulation and may create the appearance that the test becomes more sensitive. However, for chemicals that prolong

the plateau of the action potential, QT-liability may falsely incriminate safe chemicals (see non-torsadogenic QT prolongation in Section 2 above). Indeed, *torsade de pointes* "may be less related to degree of QT prolongation than to drug effects on transmural dispersion or variability of repolarization" (Kannankeril and Roden, 2007).

### 7.9. Safety margins

Any chemical will become toxic at a certain concentration, but concentrations far enough below the threshold for toxicity can provide an opportunity for safe use as a drug. With respect to disturbances of repolarization, no drug is known to be torsadogenic when its safety factor (ratio of lowest concentration that induces TRIaD to upper therapeutic plasma concentration) exceeds 100. This can have very important consequences, which I would like to illustrate with 2 examples.

Vardenafil was reported to become potentially torsadogenic at 30 μM (Lawrence et al., 2006). This is in line with an $IC_{50}$=84 μM for hERG channels; however, vardenafil has an $IC_{50}$ for the target enzyme (PDE5) of only 0.89 nM (Shah, 2005). Vardenafil thus is endowed with a safety margin of 33,708, which is well beyond the most conservative safety margin.

Risperidone was studied blindly in October 2002 and reported to induce triangulation, reverse use dependence and instability at 10 nM, and to prolong the action potential duration at 100 nM. The effective free therapeutic plasma concentration is estimated to be between 0.6 and 1.8 nM (Redfern et al., 2003). Thus, in terms of TRIaD, risperidone has a safety margin of only around 5. Since risperidone had never been reported to produce *torsade de pointes* in patients (Redfern et al., 2003), TRIaD was therefore considered to have produced a false positive (Lawrence et al., 2006). In the clinic risperidone causes little QT prolongation (~4 ms; Stollberger et al., 2005). This may explain why only recently the first incident of *torsade de pointes* was reported (Raviña et al., 2007). However, risperidone was found to have 5 cardiac arrests per thousand person years or 1.5 times that for haloperidol (Hennessy et al., 2002). Remarkably, it was primarily at the lowest concentrations, where QT prolongation might not yet exist, that the sudden cardiac deaths occurred; in the absence of QT prolongation, TRIaD preferentially results in ventricular fibrillation (Hondeghem, 2006, 2007). Since the QT prolongation of risperidone and haloperidol was similar, the authors did not expect this result and stated that it might be "an incidental finding to be examined in future research" (Hennessy et al., 2002). Alternatively, QT prolongation as such might not be a solid predictor of proarrhythmia, and perhaps TRIaD can detect lethal proarrhythmia (not limited to *torsade de pointes*) even in the absence of QT prolongation. A similar suggestion was recently made by Kannankeril and Roden (2007).

### 7.10. Completeness

Completeness of λ and TRIaD evaluation is required to fully eliminate proarrhythmic potential of an agent: it must be devoid of triangulation AND reverse use dependence AND instability (see Fig. 6 in Hondeghem et al., 2001a) and even then should not shorten λ (Hondeghem, 2006). Although instability *nearly* always precedes *torsade de pointes*, this is not always the case (Hondeghem et al., 2001a). Thus, when testing only for instability of action potential duration, then one might not correctly detect the proarrhythmic potential of an agent. For example, moxifloxacin was declared to be safe for clinical use, since it did not induce any instability of action potential duration (Thomsen et al., 2006). In contrast, all four in vitro systems tested (Lu et al., 2006) were not limited to detection of instability, and they all readily detected the proarrhythmic potential of moxifloxacin. Only moxifloxacin had a TRIaD safety ratio ~30 and would therefore be expected to cause ~0.1% proarrhythmic events in patients. A recent prospective clinical trial (Veyssier et al., 2006) found that 0.14% critical adverse events from potential cardiac origin, as validated by a scientific committee, were related to moxifloxacin. There was no instance of *torsade de pointes*, but had there been even only 1 *torsade de pointes* in only 13,578 patients, then the estimated incidence would translate to 74 *torsade de pointes* per million. Nevertheless, *torsade de pointes* has now been reported for moxifloxacin (Lawrence et al., 2006; Dale et al., 2007). To my knowledge, neither erythromycin nor telithromycin has been estimated to have similar high adverse cardiac events in a prospective clinical trial. Thus both the preclinical and clinical evidence, although both too small to yield definitive answers, suggest that moxifloxacin might be the most proarrhythmic in this series.

Although instability is the most prevalent requirement for *torsade de pointes*, triangulation and dispersion can also be essential torsadogenic identifiers (Milberg et al., 2007; Hondeghem, 2007).

Finally, instability can sometimes be latent and only become detectable upon challenge by irregular rhythm (Hondeghem et al., 2001a); this is similar to the clinic where *torsade de pointes* is mostly initiated following an arrhythmia. Thus, in addition to evaluate for TRIaD and λ, it might be best to also systematically look for instability induced by irregular stimulation and rhythm disturbances.

## 8. Drug safety research laboratories

From the above it is clear that preclinical evaluation of drug safety with respect to cardiac electrophysiology is a highly complex endeavor. It must include evaluation of triangulation, reverse use dependence, instability (including under challenge of rhythm disturbances), dispersion (spatial and temporal), conduction velocity (including its recovery kinetics), effective refractory period, and action potential duration. Only when these studies are carried out under strict application of Good Laboratory Practice, with verification of actual drug concentrations, full equilibration and application of appropriately powered statistical analyses in sufficient experiments, can one expect to obtain a reliable prediction of clinical proarrhythmia. Therefore, preclinical evaluation of proarrhythmic risks might best be done in multidisciplinary laboratories, using accepted common protocols and state-of-the-art methods for testing new

drug candidates. It might be best to study these candidates blindly together with a reference compound and a blank.

Such laboratories might eliminate some of the errors and abuses described here. Use of standardized experimental protocols in standardized animal models would be desirable, as long as additional explorations can be included so as not to strangulate but stimulate experimental progress. Drugs should be studied in adequately powered randomized and blinded studies, using standardized analyses methods perhaps from a central core facility. An excellent proposal for such consortiums was recently detailed by Bolli et al. (2004).

## 9. Conclusion

1. In various preclinical setups TRIaD and $\lambda$ are powerful proarrhythmic detectors.
2. QT prolongation as such is not, as it provides false positives as well as false negatives.
3. Only when adhering strictly to validated criteria can evaluations yield reliable safety data, i.e., devoid of false positives as well as false negatives. Abusing TRIaD, by poor study design, conduct or misrepresentation of data, confounds significance for clinical repercussions and elicits confusion regarding important drug effects.
4. Establishing a preclinical research consortium for cardiac drug safety to assure the use of (1) standardized experimental protocols; (2) standard animal models; (3) appropriately powered statistical analyses; (4) correct testing and reporting, should be considered to eliminate some of the investigator errors and abuses outlined in this paper.

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