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REVIEW

Literature-based evaluation of four 'hard endpoint' models for assessing drug-induced torsades de pointes liability

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In safety pharmacology, a number of preclinical models for detecting drug-induced proarrhythmia liability have been recently introduced that utilize hard endpoints: early after depolarziations (EADs), torsades de pointes (TdP) or both as the principal biomarker. To explore the validity of four of the most common of these models, (the isolated canine/rabbit left ventricular wedge preparation, the isolated rabbit heart, the methoxamine-pretreated anaesthetized rabbit and the complete, chronic AV-blocked (CAVB) dog (conscious and anaesthetized), the present article reviews published data sets for three drugs with recognized and different human TdP liabilities (cisparide, terfenadine and moxifloxacinin). Finally, this review considers the value of inclusion of analysis of beat-to-beat variability of repolarization (BVR) in TdP liability testing to improve sensitivity and specificity.
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Abbreviations: BVR, beat-to-beat variability of repolarization; CAVB, complete, chronic atrioventricular block; EADs, early afterdepolarizations; HERG, α-subunit of the protein complex responsible for I_{Kr}; I_{Kr}, the rapid component of the delayed rectifier current; I_{Ks}, the slow component of the delayed rectifier current; I_{Na}, sodium current; MAPD, monophasic action potential duration; STV, short-term variability; STV_{QT}, short-term variability of the QT; TdP, torsades de pointes; $T_{peak}-T_{end}$, the interval between the peak and the end of the T-wave

Introduction

Cardiovascular safety assessment of new compounds includes the evaluation of the risk for proarrhythmic effects, particularly the risk for drug-induced torsades de pointes (TdP). Many tests have been utilized to assess TdP liability, and the majority use surrogate biomarkers such as the threshold concentration or IC_{50} for block of the rapid component of the delayed rectifier current (I_{Kr}) in cell lines, the threshold concentration or IC_{50} for increasing the duration of the action potential in ventricular cells and the threshold dosage necessary to elicit a specified minimum prolongation of QT interval in normal, conscious animals (Belardinelli *et al*., 2003; ICH-S7B, 2005; Thomsen *et al*., 2006a).

With the realization that these surrogate biomarkers have major limitations (Belardinelli *et al*., 2003; Thomsen *et al*., 2006a), the academia and the industry have made an enormous effort to create (animal) models that better predict the TdP liability of (new) drugs.

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In this review, four models that utilize either TdP itself or a closely linked variable (early afterdepolarziations; EADs) as the principal biomarker, have been examined. These are the isolated canine/rabbit left ventricular wedge preparation, the isolated rabbit heart, the methoxamine-pretreated anaesthetized rabbit and the complete, chronic AV-blocked (CAVB) dog (conscious and anaesthetized).

To explore the validity of these models, three drugs with documented TdP liability in humans have been chosen for interrogation, and the literature concerning their activities in the four models has been examined (see Tables 1–3). Typically, for drugs that may have undergone extensive clinical exposure before TdP liability was recognized, some of the quinolone antibiotics evoke a low incidence of TdP in humans of 0.3–27 in 10 million prescriptions for ciprofloxacin and gatifloxacin, respectively. However, TdP has not been documented with moxifloxacin use (Frothingham, 2001).

Refining present models is an ongoing process. Interrogating a model with electrophysiological parameters, and deriving numerical outputs have been explored as approaches over many years (see review Thomsen *et al*.,

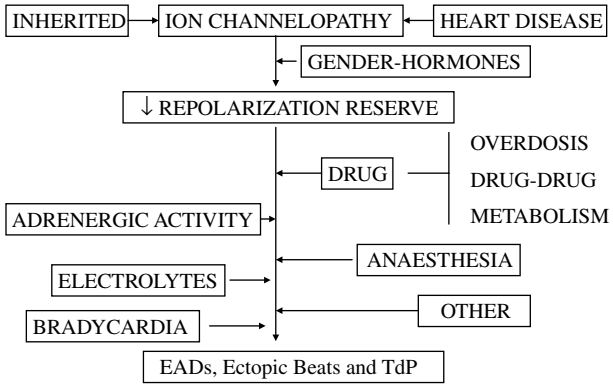

Figure 1 An overview that illustrates the events that precede the arrhythmic outcome in individuals with a reduced-repolarization reserve. The ion channelopathy (top) that is either inherited, acquired or both results in a diminished repolarization reserve. Additional hits are required, alone but most often in combination, to initiate arrhythmias (bottom). One of these (final) hits may be the administration of a drug.

2006a). In the present article, some consideration is given to a more recent derivation, beat-to-beat variability of repolarization (BVR; Thomsen *et al*., 2004).

TdP liability testing models: the need for additional biomarkers

Over the years, the two most extensively used non-human species for *in vivo* evaluation of drug-induced TdP are the dog and the rabbit. The first stage in model validation has generally involved demonstration of TdP (or EADs) in response to drugs that are known to block I_{Kr}. More recently, evidence has been provided that the block of the slow component of the delayed rectifier current (I_{Ks}) may predispose dogs to TdP under circumstances of adrenergic stimulation (Gallacher *et al*., 2007). Subsequent validation requires the demonstration that non-cardiac drugs with low but real human TdP liability can be detected as TdP liable in the model. Testing of such drugs at around the normal clinical therapeutic dose will not normally result in EADs or TdP. In fact, this holds true even for drugs with strongly established TdP liability, including the class III antiarrhythmics, sotalol and dofetilide, whose clinical TdP incidence has been described to be in the order of 1–5%. Thus, if TdP itself (or EADs) is used as the endpoint in the liability test, either the drug concentration must be increased considerably (perhaps to levels well beyond those ever likely to be encountered during clinical use—except perhaps after overdosage), or the model must be adulterated to heighten its sensitivity by adding presumed predisposing factors such as hypokalaemia, α-adrenoceptor agonism and so on (Vos *et al*., 2001; Belardinelli *et al*., 2003; Thomsen *et al*., 2006a).

There are clinical parallels, as rare event liability may manifest most commonly under the circumstances of predisposition (Figure 1). Clinically, predisposition may be caused by female gender, an inherited channelopathy, electrolyte imbalance heart disease, factors as yet unidentified, or a combination thereof. Channelopathy may reduce the cardiac repolarization reserve and increase the susceptibility for drug-induced TdP. From a drug safety perspective, a rare combination of predispositions may lower the threshold for drug-induced TdP in an individual, with combinations of off-target pharmacology having the greatest liability. Recently, Lengyel *et al*. (2007) demonstrated that combined administration of I_{Kr} and I_{Ks} blockers reduced repolarization reserve and elicited TdP in normal dogs, whereas the sole administration of either did not cause TdP.

For the intact rabbit *in vivo*, the best-known predisposing factor for TdP is pretreatment with the α-adrenoceptor agonist, methoxamine (Carlsson *et al*., 1990) with this agent, TdP can be reproducibly induced in the majority of anaesthetized rabbits (anaesthesia being a second predisposing factor) after continuous administration of class III drugs such as clofilium or almokalant. How α-receptor agonism predisposes the rabbit heart is not clear. An elevation in ventricular intracellular calcium concentration and a reflex bradycardia due to the increase in blood pressure are the two mechanisms that have been considered.

In denervated isolated rabbit hearts, numerous predisposing factors have been identified: bradycardia (complete AV-block), hypokalaemia, anemone toxin to activate the sodium current (I_{Na}), and interruption of cardiac pacing to extend diastole. In this preparation, there is a quantified risk assessment available: the SCREENIT system, developed by Hondeghem *et al*. (2003).

In the rabbit wedge preparation, bradycardia is used as a predisposing factor, created by very slow pacing (1000–4000 ms cycle length versus the physiological cycle length of 200–250 ms in this species). In this preparation, a TdP liability score system has been validated (Chen *et al*., 2006; Liu *et al*. 2006; Lu *et al*., 2007), which integrates relative changes in QT-interval, the interval between the peak and the end of the T-wave ($T_{peak}-T_{end}$) and the occurrence of EADs and TdP. The latter is the highest scoring variable in the evaluation. The canine wedge has similar characteristics.

In intact dogs, reproducible TdP induction with (class III) drugs is seen in dogs that have intervention-induced AV ablation and ventricular electrical remodelling: the CAVB dog (van Opstal *et al*., 2001). Testing has been performed both in anaesthetized as conscious conditions.

Evaluation of models

The dose dependence of TdP biomarker effects of cisapride, terfenadine and moxifloxacin have been compared for the four tests systems described above, using published data. A major point of concern is the maximal dosage that has been tested in order to provide evidence that the model is sensitive enough to identify a torsadogenic drug. Whereas in normal, non-remodeled, hearts a safety margin of 30x the clinical effective dose has been suggested as threshold for designating a drug as TdP liable (Redfern *et al*., 2003), this margin may be inappropriately high in predisposed conditions, including the conditions applied to generate the four tests systems under present scrutiny. Therefore, the relevant threshold concentrations for eliciting positive outcomes (EADs or TdP) have been included in the data presentation (Tables 1–3).

Table 1 Cisapride

	Concentrations	*TdP (EADs)*	*Conditions/ references*
LV Wedge			
Canine	0.2 μM	2/6	**Pacing-induced TdP** DiDiego *et al.*, 2003
Rabbit	0.1 μM	(2/4)	Chen *et al.*, 2006
	1 μM		Liu *et al.*, 2006
Isolated rabbit	1 μM	TRIAD↑	Hondeghem *et al.*, 2003
	0 μM	0/6 (0/7)	Low K
	0.3 μM	0/7 (4/7)	Kii and Ito, 2002
		4/6	**Low K**
	0 μM	0/6	
	0.03 μM	0/6	Wu *et al.*, 2006
		6/6	**ATX pretreated**
Methox rabbit	3 μM kg^{-1}	2/6	Carlsson *et al.*, 1997
	1.4 mg kg^{-1}	3/6	Kimura *et al.*, 2007
CAVB awake L	1 mg kg^{-1}	1/6	
high dose	10 mg kg^{-1}	6/6	Sugiyama *et al.*, 2002
CAVB anaesthetized	1 mg kg^{-1}	5/6	Winckels-Vos, unpublished

Abbreviations: CAVB, complete, chronic AV-blocked; EADs, early after depolarziations; TdP, torsades de pointes.
Between brackets, EAD incidence has been summarized, whereas the other values indicate incidence of TdP. The bold words indicate that additional hits have been provided.

Table 2 Terfenadine

	Concentrations	*TdP (EADs)*	*References*
LV wedge	$<100 \times C_{max}$	↑ *Score (No)*	Liu *et al.*, 2006
Rabbit			
Isolated rabbit	10 μM	TRIAD↑ 1/11 VT	Hondeghem *et al.*, 2003
Methox rabbit	0.3 mg kg^{-1}	0/7	Lu *et al.*, 2000
	0.75 μM	0/7	Batey and Coker, 2002
CAVB awake L	3 mg kg^{-1}	1/6	
high dose	30 mg kg^{-1}	5/6	Takahara *et al.*, 2006

Abbreviations: CAVB, complete, chronic AV-blocked; EADs, early after depolarziations; TdP, torsades de pointes; TRIAD, score based upon Triangulation, Reverse-use dependency, Instability and Dispersion; VT, ventricular tachycardia.
Between brackets EAD incidence has been summarized, whereas the other values indicate incidence of TdP.

Cisapride

Cisapride was withdrawn from the market in the United States and is listed in Woosley's category as most highly TdP liable (category 1): 'drugs with risk of TdP' (Woosley, 2008). Oral administration of 5–10 mg cisapride will result in a plasma concentration of 0.1–0.15 μM of which >95% is protein bound. In *in vitro* TdP liability tests without predisposing factors, this drug has been positively identified as causing either EADs or TdP at high concentrations (Table 1).

At lower, clinically relevant concentrations (<1 μM), TdP liability 'hits' were detected only when additional predisposing factors were introduced, such as abrupt pacing rate changes, hypokalaemia or anemone toxin pretreatment. This includes the study of Chen *et al*. (2006), where EADs were observed at 0.1 μM in the rabbit wedge (predisposed by extreme bradycardia). The *in vivo* models among the four under consideration provided similar data. A TdP liability was detected at clinically relevant dosages of 1 mg kg^{-1} orally (TdP incidence: 1/6 in the conscious CAVB dog). Higher dosages (<10×) reproducibly and consistently induced TdP in the majority of dogs.

Terfenadine

Terfenadine is also listed in Woosley's category 1. Administration of the drug registered a TdP liability hit in the rabbit left ventricle wedge and evoked ventricular tachycardia in the isolated rabbit heart (Table 2), although in the wedge, no EADs were seen (the hit came from a derivation—the wedge TdP liability score—so, strictly speaking, in the context of this article in which EADs and TdP are the hard endpoints under scrutiny, it was a 'miss'). It was identified as TdP liable in the CAVB dog at a clinically relevant dose (1/6), but not in the methoxamine-pretreated rabbit. Of interest is the observation, in the canine right ventricular wedge, that terfenadine (5 μM) caused predominantly Brugada syndrome outcomes, suggesting that its TdP liability may be linked to sodium-channel blockade and slowed conduction (DiDiego *et al.*, 2002). Impaired conduction (an increase in QRS width) with this drug has also been noted in anaesthetized rabbits (Batey and Coker, 2002) and in the isolated heart (Kii and Ito, 2002). Therefore, the mechanism of TdP liability of terfenadine may differ from that of cisapride and moxifloxacin.

Moxifloxacin

Moxifloxacin, a clinically widely prescribed antibiotic, is in the second most concerning TdP liability category of the Woosley classification: 'drug with possible risk for TdP' (Woosley, 2008). This drug is known to consistently prolong the human QT interval by 5–10 ms after an oral dosage of 400 mg kg^{-1} leading to a plasma concentration between 3–6 μM with 50% protein binding. In rabbit and canine left ventricle wedges (Table 3), moxifloxacin was detected as TdP liable (increased arrhythmia scores and EADs) only at dosages ⩾100 μM, and these hits were not consistently seen by all investigators.

In isolated rabbit hearts, EADs were first seen at 90 μM moxifloxacin (2/6 hearts), whereas TdP was seen at dosages ⩾100 μM or after pretreatment with anemone toxin. In conscious CAVB dogs, a similar picture emerged: no TdP at the low therapeutic plasma concentration (5–10 μM), but proarrhythmia seen at a dose 10× higher. In the anaesthetized CAVB dog, a dose 4× the therapeutic dosage reaching a maximal plasma concentration of 50 μM did not result in TdP, but slowed the heart rate considerably. Because moxifloxacin has predictable pharmacokinetics, the absence of TdP at clinically relevant dosages could provide a signal that this drug has no relevant TdP liability. The only discrepancy is the plasma concentration measured in the conscious CAVB

Table 3 Moxifloxacin

	Concentrations	*TdP (EAD)*	*Conditions/ references*
LV Wedge	10–100 μM	(0/5–4/5)	Chen *et al.*, 2005
Rabbit	>100 μM	↑Score (0/10)	Liu *et al.*, 2006
	300 μM	(0/7)	Lu *et al.*, 2007
Canine	100 μM	4/10	Fish *et al.*, 2005
Isolated rabbit	0 μM	0/7	
	1 μM	0/7	Wu *et al.*, 2006
	100 μM	3/8	
	1–3 μM	12/13	**ATX pretreated**
	0 μM	0/13	
	250 μM	9/13	Milberg *et al.*, 2007 **Low K**
	90 μM	(2/6)	
	30–300	0/6–1/6	Lu *et al.*, 2007
Methox rabbit		0/6	Anderson *et al.*, 2001
		0/5	Chiba *et al.*, 2004
CAVB awake L		0/4	
high 100 mg kg^{-1}	12.6 μg mL^{-1}	3/4	Chiba *et al.*, 2004
CAVB anaesthetized L	4.5 μg mL^{-1}	0/6	
High 8 mg kg^{-1}	23 μg mL^{-1}	0/6	Thomsen *et al.*, 2006b

Abbreviations: CAVB, complete, chronic AV-blocked; EADs, early after depolarziations; LV, left ventricle; TdP, torsades de pointes.
Between brackets EAD incidence has been summarized, whereas the other values indicate incidence of TdP. The bold words indicate that the additional hits have been provided.

dogs after the high dose (27 μM); a concentration too low to explain the severe TdP liability detected. This begs the question: to what dosage limit should a drug be tested? Moxifloxacin is a problem drug, in that its human TdP liability signal is so weak as to be practically irrelevant, meaning that whether or not it is a hit in model is debatable evidence when evaluating the validity of the model.

In summary, the four models have been shown to be sufficiently sensitive to allow the detection of TdP liabilities of three drugs with different perceived clinical TdP liabilities. However, to reach a useful level of sensitivity, the drugs often had to be tested at dosages that clearly exceed not only the therapeutic range but also the presently accepted safety margins for TdP liability assessment (Redfern *et al.*, 2003).

Attempts to improve sensitivity in TdP liability assessment

To improve the sensitivity and specificity in TdP liability assessment, several derived surrogate biomarkers have been examined. For this purpose, I will discuss a new surrogate parameter, BVR.

Besides sensitivity, models also have to demonstrate specificity and the ability to declare drugs safe, even if ventricular repolarization delay is detected. The term repolarization lability describes temporal dispersion of the duration of repolarization. The lability may be quantified in a number of ways: QT variability index (Berger *et al.*, 1997), instability (Hondeghem and Hoffman, 2003) and BVR (Thomsen *et al.*, 2004; van der Linde *et al.*, 2005).

In CAVB dogs, an (abrupt) increase in BVR has been shown to be associated with perceived TdP liability for many drugs (Takahara *et al.*, 2006; Thomsen *et al.*, 2006a, b, c). This increase in BVR, quantified as short-term variability (STV) of the left ventricular monophasic action potential, is measured before the first ectopic beat(s) that originate before TdP. In most instances, this BVR increase is seen well before TdP occurrence allowing the termination of the experiment before life-threatening TdP occurs. An unchanged BVR is seen when there is no proarrhythmic event observed even after administration of a drug that may prolong QT time significantly. In addition to STV, some groups have applied the BVR concept to the electrocardiogram and determining STV of the QT (STV_{QT}). Sugiyama *et al.*, 2002 measured STV_{QT} after terfenadine and after moxifloxacin in the conscious CAVB dog and demonstrated increases in STV contemporaneously with TdP. The high dose of terfenadine (Table 2) induced TdP at 4–6 weeks of CAVB but not at 2 weeks, when presumably ventricular remodelling was not yet completed (Takahara *et al.*, 2006). At 2 weeks, STV_{QT} remained unchanged (5.4 ± 0.4 vs 5.1 ± 0.2 ms), whereas at 6 weeks, STV_{QT} increased (from 5.2 ± 0.2 to 7.2 ± 0.4 ms, $P<0.05$). Similar results were obtained with moxifloxacin (Thomsen *et al.*, 2006b), whereby in anaesthetized dogs, STV_{QT} was unchanged (2.4 ± 1.7 vs 2.6 ± 1.0 ms) when no TdP occurred in comparison to conscious dogs (4.5 ± 0.8 to 5.9 ± 0.6 ms, $P<0.05$) in which TdP occurred in three out of four dogs (Table 3). Thus, the observation of unchanged BVR may have the potential to identify safe drugs. Further testing, however, is warranted to prove this concept, not only in CAVB dogs but also in the other TdP liability test models. The first (positive) attempts have been made in the isolated rabbit heart (Wu *et al.*, 2006) and in the conscious unadulterated dog (Lengyel *et al.*, 2007).

Conclusion

The sensitivity of TdP liability models can be enhanced to such a level that drug-induced EADs or TdP can be identified. The dosages necessary to achieve a hit, however, are often many times the clinical effective dose and therefore may jeopardize (reasonable) the identification of safe drugs. Inclusion of surrogate biomarkers such as BVR in the integrated risk assessment may have the potential to identify drug-induced TdP liability at more clinically relevant dosages.

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Conflict of interest

The author states no conflict of interest.

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