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REVIEW

Sensitive and reliable proarrhythmia *in vivo* animal models for predicting drug-induced torsades de pointes in patients with remodelled hearts

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As an increasing number of non-cardiac drugs have been reported to cause QT interval prolongation and torsades de pointes (TdP), we extensively studied the utility of atrioventricular (AV) block animals as a model to predict their torsadogenic action in human. The present review highlights such *in vivo* proarrhythmia models. In the case of the canine model, test substances were administered p.o. at conscious state >4 weeks after the induction of AV block, with subsequent Holter ECG monitoring to evaluate drug effects. Control AV block dogs (no pharmacological treatment) survive for several years without TdP attack. For pharmacologically treated dogs, drugs were identified as high, low or no risk. High-risk drugs induced TdP at 1–3 times the therapeutic dose. Low-risk drugs did not induce TdP at this dose range, but induced it at higher doses. No-risk drugs never induced TdP at any dose tested. Electrophysiological, anatomical histological and biochemical adaptations against persistent bradycardia-induced chronic heart failure were observed in AV block dogs. Recently, we have developed another highly sensitive proarrhythmia model using a chronic AV block cynomolgus monkey, which possesses essentially the same pathophysiological adaptations and drug responses as those demonstrated in the canine model. As a common remodelling process leading to a diminished repolarization reserve may present in patients who experience drug-induced TdP and in the AV block animals, the *in vivo* proarrhythmia models described in this review may be useful for predicting the risk of pharmacologically induced TdP in humans.
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Keywords: chronic atrioventricular block; QT interval prolongation; torsades de pointes; ECG; repolarization reserve

Abbreviations: AV, atrioventricular; ERP, effective refractory period; MAP, monophasic action potential; I_{Kr}, a rapid component of delayed rectifier K^+ currents; TdP, torsades de pointes

An increasing number of non-cardiac drugs have been reported to delay cardiac repolarization process through inhibition of a rapid component of delayed rectifier K^+ currents (I_{Kr}) in the heart, occasionally causing torsades de pointes (TdP) in some patients, although these drugs did not show pronounced QT interval prolongation during the preclinical animal experiments or clinical studies (Roden, 2005; Thomsen *et al*., 2006b). This issue has been identified as a serious public health problem, which attracted attention from the clinicians, pharmaceutical companies as well as drug regulatory authorities. In this article, we focus on (1) *in vivo* mechanism(s) of the drug-induced QT interval prolongation, leading to the onset of TdP, (2) utility and limitation of current animal models in predicting the risk of pharmacologically induced TdP in humans and (3) comparison of these animal models with existing *in vitro* and *in vivo* proarrhythmia models.

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Onset mechanisms of TdP

QT interval prolongation by drugs that can inhibit I_{Kr} usually delays phase 3 repolarization, leading to increased electrical vulnerability of the ventricles. This can include spatial dispersion of repolarization (Volders *et al*., 1998; Vos *et al*., 1998; Sugiyama and Hashimoto, 2002; Sugiyama *et al*., 2002a; Belardinelli *et al*., 2003), as shown in Figure 1. Also, QT interval prolongation by I_{Kr} blockers often complicates temporal dispersion of repolarization, leading to the onset of early afterdepolarizations and R on T type premature ventricular contractions, which trigger TdP in the presence of proarrhythmic substrates (Volders *et al*., 1998; Vos *et al*.,

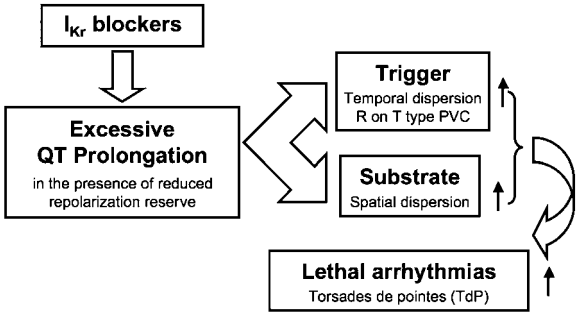

Figure 1 Onset mechanisms of TdP.

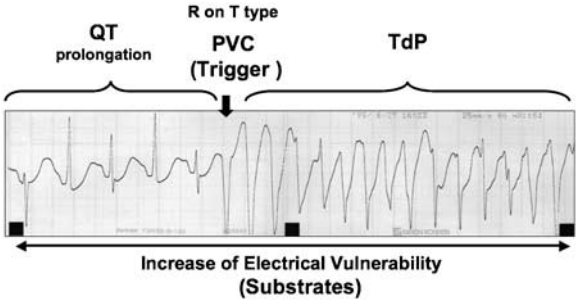

Figure 2 Typical tracing of drug-induced torsades de pointes (TdP) in patients taking antidepressant sulpiride. Significant QT interval prolongation would provide the increase of electrical vulnerability (substrate) and induce the R on T type premature ventricular contraction (PVC, trigger), leading to an onset of TdP.

1998; Sugiyama and Hashimoto, 2002; Sugiyama *et al*., 2002a; Belardinelli *et al*., 2003). As depicted in Figure 2, QT interval prolongation can provide proarrhythmic substrates and triggers in the heart, leading to the onset of TdP. This concept explains the difference between drug-induced QT interval prolongation and the onset of TdP; namely, QT interval prolongation is never lethal in and of itself, but TdP can be lethal.

Role of reduced repolarization reserve in the onset of drug-induced QT interval prolongation

Excessive QT interval prolongation by I_{Kr} blockers is thought to occur only in patients with reduced repolarization reserve (Roden, 2005). To better understand the concept of repolarization reserve, we evaluated the ability of the animal models and human data for predicting the onset of drug-induced QT interval prolongation. As summarized in Figure 3, conscious animal models usually have more repolarization reserve than healthy human volunteers, which may make the models less sensitive for detecting drug-induced QT interval prolongation (Sugiyama and Hashimoto, 2002; Sakaguchi *et al*., 2005; Takahara *et al*., 2005a, b, 2006b; Chiba *et al*., 2006). The extent of repolarization is thought to be similar in healthy human volunteers and halothane-anaesthetized dogs (Sugiyama *et al*., 1996, 1997a, b, 1999, 2001a–d, 2002c, 2003, 2004a; Sugiyama and Hashimoto, 1998; Usui *et al*., 1998; Satoh *et al*., 1999, 2000a–c, 2004, 2005; Chiba *et al*., 2000, 2004a, b; Shiina *et al*., 2000; Takahara *et al*., 2000, 2003a, b, 2004a, 2005b; Yoneyama *et al*., 2002; Yoshida *et al*., 2002a). On the other hand, both high-risk human patients and chronic atrioventricular (AV) block animals have limited repolarization reserve and elevated inward calcium current; thus, they are most susceptible to drug-induced QT interval prolongation and subsequent TdP (Chiba *et al*., 2000, 2004a; Sugiyama *et al*., 2002c, 2003; Yoshida *et al*., 2002b; Satoh *et al*., 2004; Takahara *et al*., 2004b, 2006c). In other words, most of the drugs with I_{Kr}-blocking activity do not show pronounced QT interval prolongation in a patient with normal repolarization reserve.

Utility and limitation of the halothane-anaesthetized canine model

Halothane reduces repolarization reserve through suppression of a slow component of delayed rectifier K^+ currents (I_{Ks}) and I_{Kr} in the heart and by attenuating the autonomic tone (Sakaguchi *et al*., 2005; Takahara *et al*., 2005a, b, 2006b). We have assessed many drugs with and without I_{Kr}-blocking property using this model by monitoring monophasic action potential (MAP), as schematically shown in Figure 4 (Sugiyama and Hashimoto, 1999). The results are summarized in Table 1.

Antiarrhythmic drugs with class III activity, including dofetilide, nifekalant, bepridil, sematilide, DL-sotalol and amiodarone, prolonged QT interval and the duration of MAP in the right ventricle (Satoh *et al*., 1999, 2004; Sugiyama *et al*., 2001a; Takahara *et al*., 2005b; Ishizaka *et al*., 2008). The terminal repolarization period calculated from the difference between 90% recovery of MAP duration and effective refractory period (ERP) at the same site, which reflects the extent of electrical vulnerability such as phase 3 repolarization, was shortened only by amiodarone, indicating its antiarrhythmic potential regardless of the QT interval prolongation.

Non-cardiac drugs with I_{Kr}-blocking property, including astemizole, cisapride, haloperidol, risperidone, sparfloxacin, sulpiride, gatifloxacin, moxifloxacin, terfenadine, prochlorperazine and canrenoate, also prolonged the QT interval and MAP duration (Sugiyama *et al*., 1997a, b, 2001c, 2002c, 2004a; Sugiyama and Hashimoto, 1998; Usui *et al*., 1998; Chiba *et al*., 2000, 2004a, b, 2006; Satoh *et al*., 2000b, 2005; Takahara *et al*., 2004a; Ando *et al*., 2007). The terminal repolarization period was shortened only by canrenoate, indicating its antiarrhythmic potential and also supporting lack of clinical report of TdP. Esmolol, famotidine, landiolol, levofloxacin, propranolol, sildenafil or verapamil did not affect the QT interval, MAP duration or ERP at all (Sugiyama *et al*., 1999, 2001d, 2003; Chiba *et al*., 2000; Shiina *et al*., 2000), indicating lack of risk for proarrhythmia.

Thus, this model is useful for performing qualitative and quantitative assessment of drug-induced QT interval prolongation; however, it cannot directly demonstrate the onset of TdP. In contrast, the proarrhythmia models described below do not have this limitation.

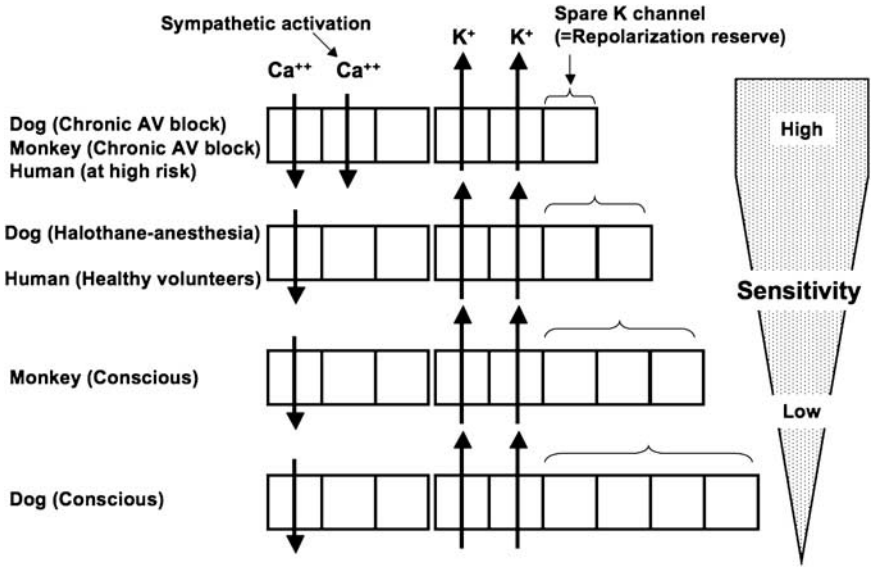

Figure 3 Comparison of the extent of repolarization reserve in animal models and humans. AV, atrioventricular.

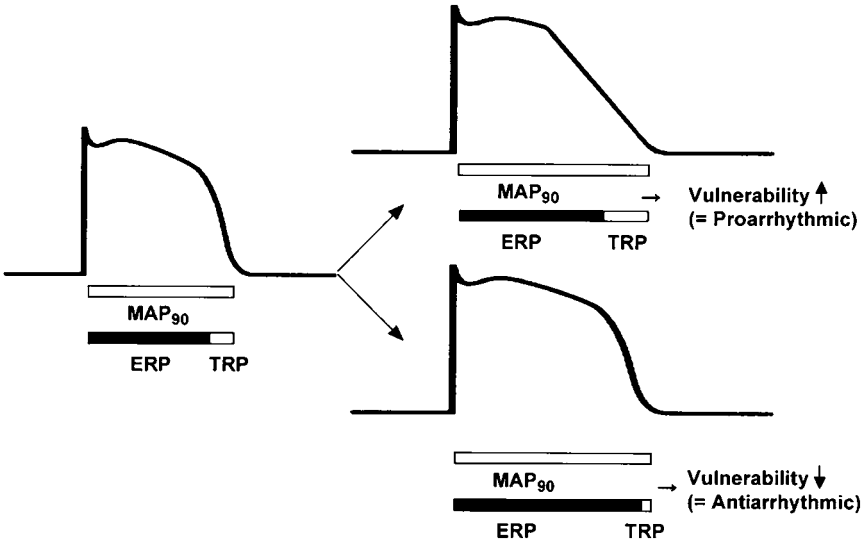

Figure 4 Schematic representation of the terminal repolarization period (TRP) calculated by the difference between the 90% recovery level of monophasic action potential duration (MAP_{90}) and effective refractory period (ERP) at the same site, which can reflect the extent of electrical vulnerability like phase 3 repolarization.

Chronic AV block dogs as a proarrhythmia model

The chronic AV block dog is a simple, but very sensitive and reliable proarrhythmia model, which can be used to evaluate the torsadogenic potential of drugs (Volders *et al.*, 1998; Vos *et al.*, 1998; Sugiyama *et al.*, 2002a). In this model, catheter ablation is used to produce AV conduction block in the canine heart. Bradycardia of 30–40 b.p.m. was observed after the induction of AV block (Sugiyama *et al.*, 2002b). As shown in Figure 5, premature ventricular contraction but not TdP was induced in <2 weeks, indicating the presence of trigger but absence of substrates. TdP was induced by cisapride in >4 weeks, suggesting the presence of both trigger and substrates. Previous studies indicated that this model was associated with macroscopic and microscopic myocardial hypertrophy, increased collagen fibre and extracellular space, downregulation of I_{Ks} channels, increased sympathetic drive promoting calcium overload and elevation of plasma atrial natriuretic peptides and angiotensin II (Volders *et al.*, 1998; Vos *et al.*, 1998; Sugiyama *et al.*, 2002a; Takahara *et al.*, 2004b, 2007a, b). These electrophysiological, anatomical, histological and biochemical adaptations against persistent bradycardia-induced chronic heart failure may predispose the chronic AV block dog to drug-induced QT prolongation with enhanced risk of re-entry and early afterdepolarization, leading to the onset of TdP. Thus, the

Table 1 Electrophysiological profile of drugs with or without I_{Kr} inhibitory property assessed by the halothane-anaesthetized canine model

MAP_{90} (QT)	*ERP*	*TRP*	*Possible profile*	*Drugs*	*i.v. dose*	*Pharmacological effect*	*References*
Prolong	Prolong	Prolong	Proarrhythmic	Astemizole	$\geqslant 0.3\,mg\,kg^{-1}$	H_1 blocker	Sugiyama *et al.* (1997a, b)
				Cisapride	$\geqslant 0.1\,mg\,kg^{-1}$	Prokinetic	Sugiyama and Hashimoto (1998)
				Dofetilide[a]	$\geqslant 0.01\,mg\,kg^{-1}$	Antiarrhythmic	Satoh *et al.* (1999)
				Haloperidol	$\geqslant 0.3\,mg\,kg^{-1}$	Antipsychotic	Satoh *et al.* (2000b); Sugiyama *et al.* (2001c)
				Nifekalant[a]	$\geqslant 0.3\,mg\,kg^{-1}$	Antiarrhythmic	Satoh *et al.* (2004)
				Risperidone	$\geqslant 0.3\,mg\,kg^{-1}$	Antipsychotic	Ando *et al.* (2007)
				Sparfloxacin	$\geqslant 3\,mg\,kg^{-1}$	Antibiotic	Chiba *et al.* (2000)
				Sulpiride	$20\,mg\,kg^{-1}$	Antidepressant	Sugiyama *et al.* (2002c)
		No change	Proarrhythmic	Bepridil[a]	$\geqslant 0.3\,mg\,kg^{-1}$	Antiarrhythmic	Ishizaka *et al.* (2008)
				Gatifloxacin	$3\,mg\,kg^{-1}$	Antibiotic	Chiba *et al.* (2004a)
				Moxifloxacin	$3\,mg\,kg^{-1}$	Antibiotic	Chiba *et al.* (2004a)
				Sematilide[a]	$\geqslant 0.3\,mg\,kg^{-1}$	Antiarrhythmic	Takahara *et al.* (2005b)
				DL-Sotalol[a]	$\geqslant 0.3\,mg\,kg^{-1}$	Antiarrhythmic	Ishizaka *et al.* (2008)
				Terfenadine	$3\,mg\,kg^{-1}$	H_1 blocker	Usui *et al.* (1998); Takahara *et al.* (2004a)
				Prochlorperazine	$3\,mg\,kg^{-1}$	Antipsychotic	Satoh *et al.* (2005)
		Shorten	Antiarrhythmic	Amiodarone[a]	$3\,mg\,kg^{-1}$	Antiarrhythmic	Sugiyama *et al.* (2001a)
				Canrenoate	$100\,mg\,kg^{-1}$	Diuretic	Sugiyama *et al.* (2004)
No effect	No effect	No effect	No risk for TdP	Esmolol	$0.3\,mg\,kg^{-1}\,min^{-1}$	β blocker	Sugiyama *et al.* (1999)
				Famotidine	$10\,mg\,kg^{-1}$	H_2 blocker	Sugiyama *et al.* (2003)
				Landiolol	$0.3\,mg\,kg^{-1}\,min^{-1}$	β blocker	Sugiyama *et al.* (1999)
				Levofloxacin	$3\,mg\,kg^{-1}$	Antibiotic	Chiba *et al.* (2000)
				Propranolol	$0.3\,mg\,kg^{-1}$	β blocker	Shiina *et al.* (2000)
				Sildenafil	$3\,mg\,kg^{-1}$	PDE5 inhibitor	Sugiyama *et al.* (2001d)
				Verapamil	$0.3\,mg\,kg^{-1}$	Calcium blocker	Shiina *et al.* (2000)

Abbreviations: ERP, effective refractory period; I_{Kr}, a rapid component of delayed rectifier K^+ currents; MAP_{90}, monophasic action potential at 90% recovery level; PDE: phosphodiesterase; TdP, torsades de pointes; TRP: terminal repolarization period ($=MAP_{90}$–ERP) .
[a]Antiarrhythmic drugs with class III activity.

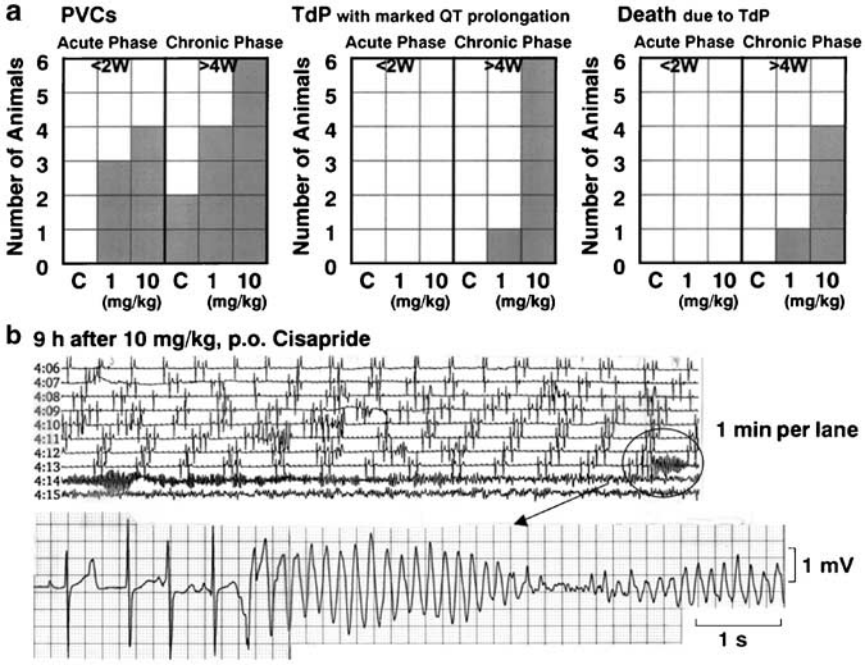

Figure 5 Comparison of onset of cisapride-induced torsades de pointes (TdP) between <2-weeks and >4-weeks model after atrioventricular block. Cisapride at 1–10 times of clinically relevant maximum daily dose was orally administered in conscious state to each model. ECG was monitored for >20 h using Holter ECG recording system. (**a**) Summary of the results. (**b**) Compressed and continuous ECG image of a dog experiencing TdP (from Sugiyama *et al.*, 2002a).

Table 2 Risk stratification of QT-prolonging drugs assessed by the chronic atrioventricular block dogs

Possible risk	*Drugs*	*I_{Kr} inhibition*	*Incidence[a] TdP*	*Drug doses ~1–3 × CD*	*Incidence[a] TdP*	*Drug doses ~10–30 × CD*	*References*
Non-cardiovascular drugs							
High	Terfenadine	+	1/6	3 mg kg^{-1}, p.o.	5/6	30 mg kg^{-1}, p.o.	Takahara *et al.* (2006c)
	Cisapride	+	1/6	1 mg kg^{-1}, p.o.	6/6	10 mg kg^{-1}, p.o.	Sugiyama *et al.* (2002a)
Low	Gatifloxacin	+	0/4	10 mg kg^{-1}, p.o.	2/4	100 mg kg^{-1}, p.o.	Chiba *et al.* (2004a)
	Moxifloxacin	+	0/4	10 mg kg^{-1}, p.o.	3/4	100 mg kg^{-1}, p.o.	Chiba *et al.* (2004a)
	Sparfloxacin	+	0/4	6 mg kg^{-1}, p.o.	4/4	60 mg kg^{-1}, p.o.	Chiba *et al.* (2000)
	Sulpiride	+	0/4	6 mg kg^{-1}, p.o.	1/4	60 mg kg^{-1}, p.o.	Sugiyama *et al.* (2002c)
No	Famotidine	−	0/4	1 mg kg^{-1}, i.v.	0/4	10 mg kg^{-1}, i.v.	Sugiyama *et al.* (2003)
	Levofloxacin	−	0/4	6 mg kg^{-1}, p.o.	0/4	60 mg kg^{-1}, p.o.	Chiba *et al.* (2000)
Antiarrhythmics							
High	D-Sotalol	+	1/4	3 mg kg^{-1}, p.o.	4/4	30 mg kg^{-1}, p.o.	Takahara *et al.* (2007a)
Low	Nifekalant	+	0/5	3 mg kg^{-1}, p.o.	5/5	30 mg kg^{-1}, p.o.	Satoh *et al.* (2004)
	Bepridil	+	0/4	3 mg kg^{-1}, p.o.	3/4	30 mg kg^{-1}, p.o.	Takahara *et al.* (2007a)
	Sematilide	+	0/4	3 mg kg^{-1}, p.o.	3/4	30 mg kg^{-1}, p.o.	Yoshida *et al.* (2002b)
No	Amiodarone	+	0/4	3 mg kg^{-1}, p.o.	0/4	30 mg kg^{-1}, p.o.	Yoshida *et al.* (2002b)

Abbreviations: CD, clinically relevant drugs doses; I_{Kr}, a rapid component of delayed rectifier K^+ currents; TdP: torsades de pointes.
[a]No. of animals that showed TdP/no. of experiments.

remodelling process in the AV block canine heart may mimic the pathophysiology in human patients at risk of drug-induced TdP.

We have assessed proarrhythmic effects of non-cardiac drugs and antiarrhythmics possessing common I_{Kr}-blocking property using the chronic AV block dog as a model. The results are summarized in Table 2. The p.o. doses of drugs used for the chronic AV block animal models were calculated simply based on clinically relevant, potentially maximum daily doses in milligram per kilogram basis for non-cardiac drugs, whereas those were obtained based on single oral doses for antiarrhythmics. Assessment was carried out at >4 weeks after the induction of AV block using Holter ECG recording to monitor drug-induced effects. Terfenadine, cisapride and D-sotalol induced TdP in all animals at 10–30 times the clinically relevant dose (Sugiyama *et al.*, 2002a; Takahara *et al.*, 2006c, 2007a). TdP was also induced in some animals at 1–3 times the clinically relevant dose of each drug. Gatifloxacin, moxifloxacin, sparfloxacin, sulpiride, nifekalant, bepridil and sematilide induced TdP in some animals at 10–30 times the clinically relevant dose, but did not induce TdP in any animal at 1–3 times the clinically relevant dose (Chiba *et al.*, 2000, 2004a; Sugiyama *et al.*, 2002c; Yoshida *et al.*, 2002b; Satoh *et al.*, 2004). Famotidine, levofloxacin or amiodarone did not induce TdP in any animals at any dose tested (Chiba *et al.*, 2000; Yoshida *et al.*, 2002b; Sugiyama *et al.*, 2003).

Because the chronic AV block model simulates a patient who is most sensitive to I_{Kr} blockers (Volders *et al.*, 1998; Vos *et al.*, 1998; Sugiyama *et al.*, 2002a; Thomsen *et al.*, 2006a, b), we propose the following interpretation of these results: if TdP was induced by 1–3 times the therapeutic dose of the drug, the drug can be considered to have high risk of proarrhythmia. If TdP was induced by 10–30 times the therapeutic dose, but not by 1–3 times the therapeutic dose, the drug has low risk (that is, practically no risk for most of the patients). If TdP was not induced by either dose, the drug has no risk. The dosage to test will represent the clinically relevant dose, when using such a highly susceptible animal model. In other words, onset of TdP in the chronic AV block model could become a reliable surrogate for all TdP risks of drugs in human. Thus, the chronic AV block canine model can provide important information concerning the proarrhythmic risk of clinically relevant drugs.

It should be also noted that different risks for producing TdP are accepted for different classes of drugs. For example, a higher risk may be acceptable for an anti-neoplastic used for a life-threatening disease than for an anti-histamine used for a rhinorrhoea; thus, practical safety margin should be judged by taking into account the risk–benefit balance in addition to the extent of torsadogenic potential. Additional use of pharmacokinetic information such as C_{max}, T_{max} and area under the concentration–time curve together with the information regarding the tissue drug distribution may improve the sensitivity and reliability of this model for estimating the safety margin of drugs.

A novel proarrhythmia model: the chronic AV block monkey

We have developed a novel *in vivo* proarrhythmia model of drug-induced TdP using cynomolgus monkeys (Satoh *et al.*, 2006). Monkeys were sedated under general anaesthesia and the AV node was ablated; animals were monitored by intracardiac electrograms, in essentially the same manner as for the canine model. Bradycardia of 50–60 b.p.m. (faster than in the canine model) was observed after the induction of AV block. Similar to the canine model, electrophysiological, anatomical, histological and biochemical adaptations suggest predisposition to drug-induced TdP (Sugiyama *et al.*, 2007). The effects of drugs listed in Table 2 on the ECG of chronic AV block monkeys were assessed at conscious state >12 weeks after the induction of AV block (Figure 6). We obtained essentially

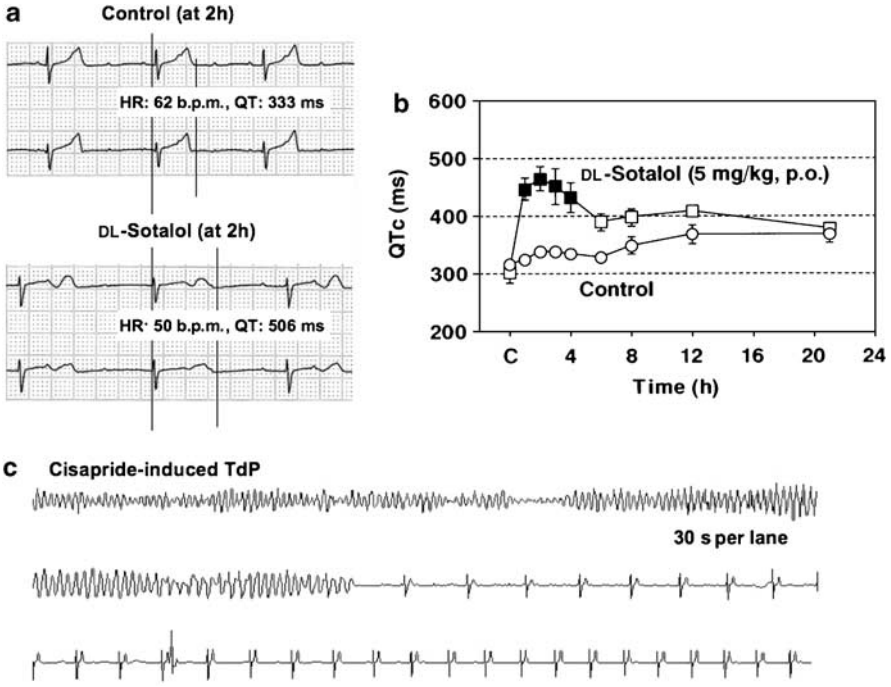

Figure 6 Summary of the effects of a rapid component of delayed rectifier K^+ currents (I_{Kr}) blockers on the ECG of the chronic atrioventricular block monkey model. (**a**) Typical ECG tracings showing DL-sotalol (5 mg/kg, p.o.)-induced QT interval prolongation. (**b**) Summary of the effects of DL-sotalol or vehicle on QTc ($n=6$). (**c**) Typical ECG tracings showing cisapride (5 mg/kg, p.o.)-induced torsades de pointes (TdP) and its spontaneous termination.

the same results for each drug as those observed in the canine model except for terfenadine (Takahara *et al.*, 2006a).

Indeed, terfenadine (30 mg/kg, p.o.) neither prolonged QT interval nor induced TdP at all in the AV block monkey model ($n=5$). I_{Kr} inhibition by terfenadine is reported to depend largely on its unchanged form, which is extensively metabolized by hepatic CYP3A4 in primates unlike dogs (Ando *et al.*, 2005). Also, ketoconazole was shown to significantly increase the plasma concentration of orally administered terfenadine through inhibition of hepatic CYP3A4 metabolic pathway in normal cynomolgus monkeys (Ando *et al.*, 2005). On the basis of this previous knowledge, we administered ketoconazole (100 mg/kg, p.o.) to the AV block monkeys 1 h before the terfenadine treatment (30 mg/kg, p.o.), and found that in the presence of ketoconazole, terfenadine significantly prolonged QT interval, resulting in the onset of TdP in all animals ($n=4$).

Thus, this novel monkey proarrhythmia model is sufficiently reliable to predict the onset of TdP. It should be noted that the monkey model has significant advantages over previous TdP models, because TdP always terminates spontaneously in monkeys (Figure 6c); thus, no monkeys died during the study. This makes it possible to compare the effects of multiple drugs in the same animals. The monkey model may also prove useful when the test article produces emesis in other species, or metabolites and/or metabolic pathways of a drug are unique to primates. In addition, experiments with monkeys require smaller amounts of drugs for testing, because their body weight/size is small (that is, approximately 3 kg).

Advantage and disadvantage of existing proarrhythmia models in comparison with the AV block animal models

Other than the chronic AV block animals, there are some *in vitro* and *in vivo* models that have been suggested to be of value in assessing drug-induced proarrhythmia liability; namely, the isolated arterially perfused left ventricular wedge preparation (*in vitro*), the Langendorff-perfused rabbit heart (*in vitro*) and the methoxamine-sensitized rabbit (*in vivo*). A measurable incidence of TdP in these models and human is summarized in Table 3.

The isolated arterially perfused left ventricular wedge preparation provides important information about the transmural repolarization heterogeneity, which serves as a reliable marker of proarrhythmia liability (Yan and Antzelevitch, 1998). TdP-like arrhythmia can initiate spontaneously, which is more frequently seen in the rabbit than in the canine preparation when exposed to QT-prolonging drugs (Yan *et al.*, 2001).

In the Langendorff-perfused rabbit heart model, bradycardia is induced by AV nodal ablation and the heart is paced at a constant slow rate (Eckardt *et al.*, 1998). Under such experimental conditions, perfusion with drugs that significantly delay ventricular repolarization readily results in the induction of early afterdepolarizations followed by onset of TdP. Repolarization abnormalities, including beat-to-beat variability, phase 3 prolongation and reverse use dependence, and related arrhythmias are most often documented by the simultaneous recording of ECG and MAP (Hondeghem *et al.*, 2001). There are numerous background data of

Table 3 A measurable incidence of TdP in human and *in vivo*/*in vitro* animal models

Drugs	*Human*	In vivo *model*		In vitro *model*	
	Clinical reports	*Chronic AV block dog*	*Methoxamine-sensitized rabbit*	*Langendorff-perfused heart*	*Ventricular wedge preparation*
Astemizole	+ Woosley (2008)[a]	+ Sugiyama *et al.* (2004b); Takahara *et al.* (2007b)		+ Steidl-Nichols *et al.* (2008)	
Bepridil	+ Woosley (2008)[a]	+ Takahara *et al.* (2007a)		+ Lawrence *et al.* (2006)	
Cisapride	+ Woosley (2008)[a]	+ Sugiyama *et al.* (2002a)	+ Carlsson *et al.* (1997)	+ Lawrence *et al.* (2006); Steidl-Nichols *et al.* (2008)	+ Di Diego *et al.* (2003); Fish *et al.* (2004)
Gatifloxacin	+ Woosley (2008)[b]	+ Chiba *et al.* (2004a)	+ Chiba *et al.* (2004a)		
Haloperidol	+ Woosley (2008)[a]	+ Sugiyama *et al.* (2004b)		+ Hondeghem and Hoffmann (2003); Steidl-Nichols *et al.* (2008)	
Moxifloxacin	+ Woosley, (2008)[b]	+ Chiba *et al.* (2004a)	− Anderson *et al.* (2001); Chiba *et al.* (2004a)	+ Lu *et al.* (2006); Milberg *et al.* (2007); Steidl-Nichols *et al.* (2008)	− Chen *et al.* (2005)
Nifekalant	+ Ohashi *et al.* (2006)[a]	+ Satoh *et al.* (2004)	+ Inaba *et al.* (2008)		
Sematilide	+ Redfern *et al.* (2003)[a]	+ Yoshida *et al.* (2002b)	+ Carlsson *et al.* (1990); Brooks *et al.* (2000)		
D-Sotalol	+ Woosley (2008)[a]	+ Thomsen *et al.* (2004); Takahara *et al.* (2007a)	+ Buchanan *et al.* (1993); Farkas *et al.* (2002)	− Zabel *et al.* (1997)	+ Shimizu *et al.* (1999)
Sparfloxacin	+ Woosley (2008)[a]	+ Chiba *et al.* (2004a)	+ Anderson *et al.* (2001); Chiba *et al.* (2004a)	− Lu *et al.* (2006)	
Sulpiride	+ Sugiyama *et al.* (2002c)[b]	+ Sugiyama *et al.* (2002c)			
Terfenadine	+ Woosley (2008)[a]	+ Sugiyama *et al.* (2003); Takahara *et al.* (2006c)	− Lu *et al.* (2000); Batey and Coker (2002)	+ Hondeghem and Hoffmann (2003)	+ Di Diego *et al.* (2002)
Amiodarone	+ Woosley (2008)[a]	− Van Opstal *et al.* (2001); Yoshida *et al.* (2002b)	− Farkas *et al.* (2002)	− Zabel *et al.* (1997)	− Sicouri *et al.* (1997)
Famotidine	+ Lee *et al.* (2004)[c]	− Sugiyama *et al.* (2003)			
Levofloxacin	+ Woosley (2008)[b]	− Chiba *et al.* (2000)	− Akita *et al.* (2004)	+ Milberg *et al.* (2007)	

Abbreviations: AV, atrioventricular; TdP, torsades de pointes.
[a]Drugs that are generally accepted to have a risk of causing TdP.
[b]Drugs that in some reports have been associated with TdP and/or QT prolongation but at this time lack substantial evidence for causing TdP.
[c]Drugs that, in some reports, have been weakly associated with TdP and/or QT prolongation but that are unlikely to be a risk for TdP when used in usual recommended dosages and in patients without other risk factors (for example, concomitant QT-prolonging drugs, bradycardia, electrolyte disturbances, congenital long QT syndrome, concomitant drugs that inhibit metabolism) (based on Sugiyama *et al.*, 2002c, 2003; Yoshida *et al.*, 2002b; Satoh *et al.*, 2004; Woosley, 2008).

drug-induced repolarization delay and proarrhythmia with this model (Carlsson, 2006; Thomsen *et al.*, 2006b).

These *in vitro* models described above are devoid of the influence of metabolic, humoral and nervous systems, so direct extrapolation of the drug effects *in vitro* to those in patients is difficult even when the concentrations are similar between patient plasma and perfusion solution. Torsadogenic potential depends on interactions with metabolites, autonomic nervous system and pathophysiology of the heart, which can be explored only *in vivo* preparations. For this reason, *in vitro* preparations are used primarily for the determination of potential to produce TdP, whereas *in vivo* models are adopted to directly demonstrate the drug-induced TdP.

Methoxamine-sensitized anaesthetized rabbit model was developed in the early 1990s (Carlsson *et al.*, 1993). An elevated intracellular Ca^{2+} concentration, mediated through α_1-adrenoceptor stimulation, most likely plays a fundamental role in increasing the proarrhythmia susceptibility of this model. This *in vivo* model has been the most widely used to assess the proarrhythmic potential of drugs, as it is inexpensive, easily accessible, reproducible and sensitive (Carlsson, 2006). However, caution has to be paid for screening drugs with inherent α-adrenoceptor-blocking

properties, which may partly explain this model cannot detect proarrhythmic potentials of several drugs. Drugs have to be administered intravenously under the anaesthetic condition. Need for anaesthesia affects profoundly the electrophysiology of the heart, including autonomic tone and cardiac ion channels.

In the chronic AV block animals, proarrhythmic action of drugs can be assessed under the anaesthetic as well as conscious condition through any route irrespective of single or repeated administration. Other disease models may actually be better predictors, as few persons receiving torsadogens have third-degree AV block, and orders of magnitude more have heart failure, diabetes and hypertrophy (Hamlin, 2007), thus suggesting that models possessing such diseases propitious for development of TdP might also be valuable, and possibly less costly and time consuming.

Conclusions and perspectives

QT interval prolongation does not always induce TdP. Thus, to better predict risk of drug-induced TdP, we have developed the halothane-anaesthetized canine model and chronic AV block canine and monkey models, and analysed the risk of drug-induced proarrhythmia. When deciding the continuation of development of a potential drug, assessment by both standard repolarization assays and proarrhythmic tests may be essential. The anaesthetized model provides qualitative and quantitative assessment of drug-induced QT interval prolongation. Meanwhile, the AV block models have a pathophysiology of bradycardia-induced chronic heart failure and hypertrophy, which will provide important information on risk of future drug-induced TdP in patients with reduced repolarization reserve.

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Conflict of interest

The author states no conflict of interest.

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