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COMMENTARY

Double pharmacological challenge on repolarization opens new avenues for drug safety research

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The pharmaceutical industry is testing new potential drugs for their propensity to prolong human cardiac repolarization, and regards this as a sign of proarrhythmic risk. Many studies have dethroned the common perception that prolonged repolarization is a reliable surrogate marker for torsades de pointes (TdP) arrhythmia. Both the pharmaceutical industry and the regulatory bodies are neglecting the available proarrhythmia models. *In vitro* studies have suggested that combined pharmacological hits on repolarization will produce a superior substrate for *in vivo* proarrhythmia, compared to the single-drug assessment. By using consecutive pharmacological challenges, a simple model is proposed, in which combinatorial pharmacology is employed to provoke TdP in the conscious dog. The pharmaceutical industry interested in evaluating the proarrhythmic potential of their present and future drugs now has a simple means of doing so.
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Keywords: action potential; beat-to-beat variability of repolarization; drug research; electrophysiology; long QT; proarrhythmia; torsades de pointes; repolarization reserve

Abbreviations: I_{Kr}, the rapidly activating, delayed rectifying potassium current; I_{Ks}, the slowly activating, delayed rectifying potassium current; TdP, torsades de pointes

Cancer research has proposed a *multihit* model of oncogenesis, in which sequential damage on a cell is necessary for tumour progression and malignant transformation. Scientists exploring repolarization-dependent cardiac proarrhythmia have quickly adopted this model for the explanation of *torsadogenesis*. In this model, the healthy heart comprises surplus repolarizing capacity through overlapping mechanisms of repolarization. When one such mechanism is targeted, the remaining repolarization forces are adequate to bring the cardiac cell to a normal repolarized state within a physiologically normal time frame. Patients with such subclinical repolarization disorders receiving additional injuries on the repolarization pathway can respond by excessive QT prolongation, increased temporal and spatial dispersion of repolarization duration, leading to triggered activity and torsades de pointes (TdP), ultimately culminating in sudden death. On the other hand, a single hit in the absence of other repolarization lesions is usually not adequate to generate TdP. Thus, a physiological repolarization *reserve* exists to guard against intense perturbations, and it takes multiple hits on repolarization to disturb this system.

One frequent challenge on the repolarization reserve is created through administration of drugs that reduce a repolarizing current. A large number of pharmaceutical compounds have been associated with block of the rapidly activating, delayed rectifying potassium current (I_{Kr}) (Haverkamp *et al*., 2000). Before marketing of new pharmaceutical compounds, an extensive battery of tests is conducted to estimate the propensity to prolong human repolarization. These investigations are performed in healthy animal tissue with an intact repolarization reserve. Evidently this is problematic, as the single challenge, being the new drug candidate, may not be enough to perturb the repolarization mechanisms to a level where it is evident for the investigator. To circumvent this predicament, proarrhythmia models are available, in which the final hit is provided by the drug (Thomsen *et al*., 2006). Thus, instead of assaying for repolarization-delaying characteristics of the drug, the potential to cause TdP is evaluated. Unfortunately, these models have received little priority from regulatory authorities.

There are numerous examples from *in vitro* studies showing that a double challenge on repolarization (for example, using drugs reducing I_{Kr} in combination with the slowly activating, delayed rectifying potassium current, I_{Ks}) prolongs action potentials more than would be expected from simple summation of the two individual effects

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Table 1 *In vitro* studies where combined pharmacological blockade of I_{Kr} and I_{Ks} was assessed

Preparation	I_{Kr} *block (%)*	I_{Ks} *block (%)*	*Combined block (%)*	*Reference*
Isolated canine myocytes	+130	0	+217	Volders *et al.*, 2003
Isolated human myocytes	+41	0	+62	Jost *et al.*, 2005
Canine ventricular tissue slice	+100	+10	+160	Burashnikov and Antzelevitch, 2002
Canine papillary muscle	+25	+5	+47	Varro *et al.*, 2000
Canine papillary muscle	+50	+10	+90	Biliczki *et al.*, 2002
Canine transmural wedge	+10	+20	+110	Burashnikov and Antzelevitch, 2002
Isolated rabbit heart	+30	0	+50	So *et al.*, 2006

Abbreviations: I_{Kr}, the rapidly activating, delayed rectifying potassium current; I_{Ks}, the slowly activating, delayed rectifying potassium current.
Values shown are the relative changes in action potential duration from baseline. Some data are estimated from figures within the references and thus not accurate. All data summarize experiments performed without adrenergic stimulation.

(Table 1). The synergistic relationship of such multiple hits is applicable to the clinical situation, where the patient with a decreased repolarization reserve encounters an additional challenge and passes the threshold to potentially develop TdP. Conversely, in the individual with a sufficient repolarization reserve, the primary challenge would hardly be noticed.

In the present issue of *British Journal of Pharmacology*, Lengyel *et al*. (2007) report on the extension of these *in vitro* studies to *in vivo* animal models. Using conscious dogs, the individual and combined effects of dofetilide (an I_{Kr} blocker) and HMR-1556 (an I_{Ks} blocker) are evaluated. The authors show that this has several advantages: importantly, TdP is a potential end point, which is not available in the *in vitro* studies. Secondly, devoid of adrenergic stimulation in the *in vitro* models, I_{Ks} is quite small. By using conscious dogs, Lengyel *et al*. circumvent this problem and assesses a physiologically relevant I_{Ks}.

Interestingly, the authors do not reproduce the *in vitro* finding that combined I_{Kr} and I_{Ks} challenge prolongs the QT interval significantly more than would be expected from simple summation of the individual provocations. Rather, the QT interval in the dogs is prolonged roughly 50 ms by either I_{Kr} or I_{Ks} block, and the double challenge produced around 100 ms QT prolongation. Thus, 50 plus 50 equals 100, not 150. Why the supra-additive phenomenon from the *in vitro* studies is not reproduced in the conscious dogs is presently unclear, but could reside in different drug levels, or more likely, in the presence of adrenergic stimulation *in vivo*.

It is further worth mentioning that the temporal variability of the QT interval does reveal a synergistic drug potentiation. Short-term variability of the QT interval is hardly affected by a single challenge, while a significant increase is observed after the double hit. *In vitro* studies assessing the temporal variability of repolarization using combinatorial pharmacology are sparse. Only one report shows that delaying repolarization, indirectly by increasing the inward late-sodium current, potentiates the effect of various I_{Kr} blockers causing longer action potentials, increased variability of repolarization and increased triggering activity of the ventricles (Wu *et al.*, 2006). In this study, a supra-additive effect was seen on the variability of the action potential duration when the secondary drug caused proarrhythmia in the isolated heart.

Importantly, Lengyel *et al*. are provoking TdP in the dogs by simple administration of two consecutive drug challenges. This is a simple variation of the well-studied dog model where chronic AV block induces cardiac remodelling, where after a single hit from a proarrhythmic drug can tip the balance of repolarization and induce TdP. Although the dog with chronic AV block is characterized by more than I_{Ks} downregulation (Thomsen *et al*., 2007 and references therein), the pharmacological I_{Ks} reduction replaces the pathological remodelling and promises easy implementation of the model proposed by Lengyel *et al*. in the pharmaceutical industry. Pharmaceutical companies deciding to assess a new compound for proarrhythmic potential in addition to the propensity to delay cardiac repolarization can easily establish this model. The inconveniences of long-term animal care during the stages of cardiac remodelling is absent, as are the initial financial expenses to equip a large animal operating theatre including anaesthesia and fluoroscopy. Furthermore, the biological variation in the severity of pathology is replaced by differences in pharmacodynamics and pharmacokinetics.

Questions remaining to be answered include reproducibility in TdP induction and how the model responds to 'safe' drugs. Group-wise reproducibility in the paper by Lengyel *et al*. was between 50 and 63% depending on the order of drug administration. Serial reproducibility, using the dog as its own control, was not been assessed and this would probably require defibrillator implantation. It is interesting to observe that the QT variability is increased in proarrhythmic dogs only, confirming results from anaesthetized animals (Thomsen *et al*., 2004). Thus, both parametric and non-parametric end points are available, implying that even a borderline increase in repolarization variability in combination with the absence of arrhythmia would warrant attention. No dose-dependent information is available from Lengyel *et al*., but others have shown that both dofetilide and HMR-1556 dose-dependently prolongs the QT interval in conscious dogs (Volders *et al*., 2003; van der Linde *et al*., 2005), so it is likely that the doses can be fine tuned to meet the requirements of the investigator. A final question to be addressed is whether the I_{Kr} or the I_{Ks} blocker should be administered first. From the available data it does not seem to be a critical aspect; however, if the investigative new compound is an I_{Kr} blocker and the hypothesis is that it can serve as a final hit for induction of proarrhythmia, it would seem natural to administer the I_{Ks} blocker first. All these minor limitations can readily be solved by the pharmaceutical company choosing to embrace this model.

For years, the scientific community has argued that simple repolarization assays are not suitable to assess the proarrhythmic potential of drugs. The regulatory authorities are very reluctant in adopting this view and the industry claims that, as long as the authorities will not prioritize screening for TdP, the expense of establishing these models are too high. Now is the time for the industry to surpass regulations and explore the avenues that have been opened up for them.

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Conflict of interest

The author states no conflict of interest.

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