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COMMENTARY

Drugs and trafficking of ion channels: a new pro-arrhythmic threat on the horizon?

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Tuning of functional expression levels of the ion channels that make up the cardiac action potential (AP) is crucial for preserving correct AP duration (APD) and QTc times. Many compounds inhibit human ether-à-go-go related gene (hERG)-mediated delayed rectifier currents and thus prolong cardiac repolarization that may cause life-threatening arrhythmias like Torsades de Pointes. An increasing number of drugs are found to inhibit hERG function by a dual mechanism of short-term channel block and long-term trafficking defects that operate over different time and concentration scales. In safety screens at present used by pharmaceutical companies, the short-term effect of channel block is covered. In contrast, specific screening for long-term trafficking defects is not common, with the consequent risk of drugs that disturb trafficking entering the market. Whether that poses another pro-arrhythmic threat for the patients treated has to be determined, but is not unlikely.
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Abbreviations: AP, action potential; APD, action potential duration; hERG, human ether-à-go-go related gene; I_{Kr}, rapid delayed rectifier current carried by hERG; LQTS, long QT syndrome; QT, depolarizing–repolarizing time interval on ECG

The electrocardiogram (ECG) is the result of voltage differences generated by tens of millions of cellular action potentials (AP). In mammalian cardiac ventricular myocytes, the AP is itself based on many inward and outward currents. The principal repolarization currents are the rapidly (I_{Kr}) and slowly (I_{Ks}) activating components of the delayed rectifier K^+ current. The pore-forming subunits of channels responsible for I_{Kr} are encoded by the human ether-à-go-go related gene (hERG or *KCNH2*) and for I_{Ks} by the *KCNQ1* gene, while modulating proteins encoded by the *KCNE* family are also implicated. Mutations in these genes were initially linked to repolarization disorders (the congenital long depolarizing–repolarizing time interval on ECG (QT) syndrome, LQTS), to the occurrence of torsades de pointes arrhythmias and to sudden cardiac death, often when the repolarization reserve is maximally challenged (Roden *et al*., 2002).

The starting paradigm was that these mutations were causing malfunctioning of the specific repolarization currents ('loss of I_{Kr} function'), thereby decreasing the total current available and increasing the time necessary for repolarization to complete. More recently, it has been shown that many if not most of the LQTS2 mutations are caused by trafficking defects instead of malfunctioning of the current (Anderson *et al*., 2006). By decreasing the number of proteins that reach the sarcolemma, the number of ion channel proteins is diminished ('reduced hERG density') also leading to less total current and hence to problems in controlling repolarization.

At the other end of the spectrum of the LQTS are drugs that block specific ion channels, reducing the current and prolonging the cellular AP duration (APD) visualized on the ECG as an increase in QT time (Fitzgerald and Ackerman, 2005). These drugs have also been implicated in torsades de pointes arrhythmias and sudden cardiac death (Thomsen *et al*., 2006). The list of drugs that are pro-arrhythmic is alarmingly long and consists mostly of I_{Kr} blockers ('block of function') that have common drug binding sites within the protein structure (Stansfeld *et al*., 2007). More recently, attention has shifted to drugs that disrupt hERG protein trafficking to the cell surface membrane (for example Cordes *et al*., 2005; Kuryshev *et al*., 2005; Rajamani *et al*., 2006; Sun *et al*., 2006; Guo *et al*., 2007; Wang *et al*., 2007). An extensive screen performed by Wible *et al*. (2005) showed that approximately 40% of acute hERG blockers also affect trafficking leading to the 'reduced density' hypothesis to explain their pro-arrhythmic mechanism (Eckhardt *et al*., 2005; Hancox and Mitcheson, 2006). Of note, there are drugs that share both properties and in case of the antifungal drug ketoconazole, the contribution is equal, independent and additive as described in this issue of the *British Journal of Pharmacology* by Takemasa *et al*. (2007).

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One of the characteristics that may distinguish between the two mechanisms of action is the time at which the drug effect becomes evident. In case of 'block of function', the block appears immediately (minutes), while in the 'reduced density' hypothesis, this action may take from hours to days to occur. In the Takemasa study, the 'block of function' effect is maximal within 5 min following drug application. The 'reduced density' effect becomes apparent 8 h after treatment and mature hERG expression levels continue to decrease for up to 48 h. Another, clinically important, observation indicated that upon washout of ketoconazole, the 'block of function' was resolved within minutes, while the 'reduced density' persisted for up to 48 h.

A second characteristic difference between the two effects is the underlying molecular mechanism. As shown by Takemasa *et al*. (2008) and Ridley *et al*. (2006), 'block of function' depends on two aromatic amino-acid residues, Y652 and F656, residing in the transmembrane region S6 of the hERG channel, while 'reduced density' is independent of them. Ketoconazole affected hERG maturation and therefore impaired trafficking from ER to Golgi (Takemasa *et al*., 2008) and these authors strengthened their findings by applying fluorescence-activated cell sorting and electrophysiology to demonstrate that fewer hERG channels reach the plasma membrane. Importantly, they declined to rely on suggestive but inconclusive confocal microscopy, which inherently suffers from insufficient spatial resolution to distinguish between localization on or just below the plasma membrane. Data from different fields indicate the involvement of specific amino-acid domains and interacting accessory proteins for effective trafficking of cardiac ion channels towards the sarcolemma (Steele *et al*., 2007). As many of the congenital forms of LQT2 involve trafficking defects, analysis of the function of these mutated residues (Walker *et al*., 2007) is a first step to elucidate the mode of action by which drugs affect trafficking of cardiac ion channels.

This difference in mechanism between 'block of function' and 'reduced density' poses a fundamental question. Can long-term trafficking defects occur without short-term blockade? In the current drug testing assays, agents are considered as non-safe when they induce I_{Kr} block acutely and these compounds are often not pursued further. This may be an enormous waste, since I_{Kr} block does not necessarily result in APD/QT-lengthening or life-threatening arrhythmias *in vivo*. Nevertheless, this approach will prevent potentially unsafe drugs from entering the market. On the other hand, drugs that display only long-term effects might pass the short-term safety screens. Some of these drugs may be eliminated as a result of long-term *in vivo* screening, but only when they cause QT prolongation,

Table 1 Drugs evaluated for short and long term effects on I_{Kr}

Drug	I_{kr} *channel block*	*hERG trafficking inhibition*	*Ratio*	*Other currents*[a]	*Trafficking others*[b]	*QT-APD increase*	*Reference*
Arsenic trioxide	+	+ +	> trafficking	$I_{CaL}\uparrow$ $I_{Ks}\downarrow\leftrightarrow$ $I_{Kur}\uparrow$ $I_{K\text{-}ATP}\uparrow$ $I_{to}\leftrightarrow$ $I_{K1}\leftrightarrow$ $I_{Na}\leftrightarrow$	Kv1.5↑	Yes	Drolet *et al*. (2004) Ficker *et al*. (2004)
Celastrol	+	+ +	> trafficking	$I_{K1}\downarrow$ $I_{Kv2.1}\leftrightarrow$	Kir2.1↓	Yes	Sun *et al*. (2006)
Digitoxin/ouabain/ digoxin	−	+	> trafficking	$I_{CaL}\leftrightarrow$ $I_{Ks}\leftrightarrow$ $I_{Kur}\leftrightarrow$ $I_{K1}\leftrightarrow$	Kv1.5↔	Yes	Wang *et al*. (2007)
Dofetilide	+	Not reported		Specific I_{Kr} only	Not reported	Yes	Carmeliet (1992)
Fluoxetine	+	+	Equal	$I_{Na}\leftrightarrow$	Not reported	Yes	Rajamani *et al*. (2006); Wible *et al*. (2005)
Ketoconazole	+	+	Equal	$I_{Na}\leftrightarrow$	Not reported	Yes	Dumaine *et al*. (1998); Mok *et al*. (2005); Wible *et al*. (2005); Takemasa *et al*. (2007)
Pentamidine	−[c]	+	Trafficking	$I_{kur}\leftrightarrow$ $I_{Ks}\leftrightarrow$ $I_{to}\leftrightarrow$	Kv1.5↔	Yes	Cordes *et al*. (2005) Kuryshev *et al*. (2005) Wible *et al*. (2005)
Probucol	−	+	Trafficking	$I_{Na}\leftrightarrow$ $I_{to}\leftrightarrow$ $I_{K1}\leftrightarrow$		Yes	Guo *et al*. (2007)
Thioridazine	+ +	+	< trafficking			Yes	Drolet *et al*. (1999); Wible *et al*. (2005)

Abbreviation: APD, action potential duration; FACS, fluorescence-activated cell sorting; I_{Kr}, rapid delayed rectifier current carried by hERG; QT, depolarizing–repolarizing time interval on ECG.
[a]Effect of prolonged drug treatment on ion-current densities disregarding acute block.
[b]Trafficking as detected by biochemical or FACS-based methods.
[c]Acute block is observed at concentrations far above clinically relevant exposures only.

which is not obvious for drugs affecting multiple repolarizing and depolarizing ion currents simultaneously. Eventually, drugs may enter the clinical stage without being tested thoroughly for effects on ion channel trafficking.

To illustrate this risk, the characteristics of several drugs that have short-term and long-term effects on I_{Kr} are shown in Table 1 (see Wible *et al*. (2005) for a comprehensive screen of 100 compounds). Many drugs display both 'block of function' and a 'reduced density' effect, albeit the latter effect often occurs at lower drug concentrations. The difference in dose dependence further highlights the importance of evaluating the long-term risk for trafficking defects. Some drugs, such as pentamidine, display no direct I_{Kr} block, but clearly disrupt hERG trafficking at clinically relevant concentrations, which is accompanied by a prolonged APD (Cordes *et al*., 2005; Kuryshev *et al*., 2005) and increased QT and pro-arrhythmia (Eisenhauer *et al*., 1994; Girgis *et al*., 1997). Furthermore, other ion channels involved in cardiac AP formation are not affected, although trafficking defects for these channels have not been investigated thoroughly. Pentamidine has a reputation of curing critically ill patients with *Pneumocystis carinii* pneumonia, but making them highly vulnerable for lethal cardiac arrhythmias at the time they leave the intensive care unit (Quadrel *et al*., 1992), exactly the risk one would predict based on the 'reduced density' hypothesis. From this example, it seems mandatory for the pharmaceutical industry to start screening their compounds for altered ion channel trafficking too.

Finally, as attractive as the simplicity of ectopic expression systems like HEK cells might appear, life is more complex. Screening should take into account other ion channels that function in concert with I_{Kr}. Proof from cellular APD and animal models should be provided to validate that the observed *in vitro* effects are truly active and relevant when integrated in the cardiomyocyte AP. As stated by Takemasa *et al*. (2008), plasma protein binding of ketoconazole is nearly 99%, resulting in effective ketoconozole concentrations *in vivo* that most likely do not result in either 'block of function' or 'decreased density' effects with respect to hERG-mediated I_{Kr}. Second, there are several examples of drugs displaying blockade of many individual ion currents, but which in concert do not harm the cardiac AP or even relieve some forms of pro-arrhythmia (Verduyn *et al*., 1995; Oros *et al*., 2007). Moreover, some drugs like arsenic trioxide block some and activate other repolarizing currents (Drolet *et al*., 2004) further illustrating the necessity for drug screening in multifactor *in vitro* test systems and *in vivo* animal models. The use of pro-arrhythmic models (Thomsen *et al*., 2006) is advocated because only then a link can be established between drug-induced 'reduced density' and the actual occurrence of torsades de pointes.

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