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Perspective

hERG trafficking inhibition in drug-induced lethal cardiac arrhythmia

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ABSTRACT

Acquired long QT syndrome induced by non-cardiovascular drugs can cause lethal cardiac arrhythmia called *torsades de points* and is a significant problem in drug development. The prolongation of QT interval and cardiac action potential duration are mainly due to reduced physiological function of the rapidly activating voltage-dependent potassium channels encoded by human ether-a-go-go-related gene (hERG). Structurally diverse groups of drugs are known to directly inhibit hERG channel conductance. Therefore, the ability of acute hERG inhibition is routinely assessed at the preclinical stages in pharmaceutical testing. Recent findings indicated that chronic treatment with various drugs not only inhibits hERG channels but also decreases hERG channel expression in the plasma membrane of cardiomyocytes, which has become another concern in safety pharmacology. The mechanisms involve the disruption of hERG trafficking to the surface membrane or the acceleration of hERG protein degradation. From this perspective, we present a brief overview of mechanisms of drug-induced trafficking inhibition and pathological regulation. Understanding of drug-induced hERG trafficking inhibition may provide new strategies for predicting drug-induced QT prolongation and lethal cardiac arrhythmia in pharmaceutical drug development.

1. Introduction

The QT interval prolongation induced by non-cardiovascular drugs can lead to life-threatening polymorphic ventricular arrhythmia, *torsades de pointes* (TdP) and sudden cardiac death (Redfern et al., 2003). It is thought to be mainly due to inhibition of the rapidly activating delayed rectifier K^+ current (I_{Kr}) carried by pore-forming α-subunits encoded by the human ether-a-go-go-related gene (hERG) (Sanguinetti and Tristani-Firouzi, 2006; Vandenberg et al., 2012). Structurally diverse groups of drugs, including antihistamines, antibiotics, antipsychotics, etc. were shown to bind to specific sites (Y652 and F656) in the hERG channel pore and inhibit channel activities resulting in delayed repolarisation of cardiac action potential (Vandenberg et al., 2012). Several drugs have been removed from the market for this reason (Roden, 2004). Therefore, drug-induced QT prolongation is a major concern in safety pharmacology. The acute and direct inhibition of hERG is routinely assessed in preclinical studies in drug development, according to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines (ICH S7B, 2005). However, certain drugs clinically known to cause TdP were reported to have no direct channel blocking ability but to reduce the density of hERG channels in the plasma membrane, leading to prolonged QT intervals (Ficker et al., 2004). This reduction of hERG surface density has been also found in a variety of hERG direct blockers (Wible et al., 2005) and cannot be detected by the conventional hERG assay. Therefore, this new mechanism of proarrhythmia, drug-induced hERG trafficking inhibition, has become another important concern in cardiac safety.

2. hERG channel protein trafficking

The proper physiological functions of hERG channels are provided by the correctly folded and assembled proteins that traffic to the cell membrane (Fig. 1). First, the hERG mRNA is processed in the nucleus and then acts with ribosome to synthesise nascent polypeptide. In endoplasmic reticulum (ER) the hERG polypeptide chains are associated with cytosolic chaperones, heat shock protein Hsp70 and Hsp90, which assist in correct folding to prevent the misfolding or degradation of proteins (Ficker et al., 2003; Peterson et al., 2012; Young, 2014). Some high-affinity hERG blockers that bind to specific sites of the channel inner pore can stabilise misfolded hERG channels, thereby rescuing the trafficking defect as pharmacological chaperones (Ficker et al., 2002). After assembling into a tetramer to form a potassium channel α-subunit, the still-immature channel is trafficked from the ER to the Golgi by

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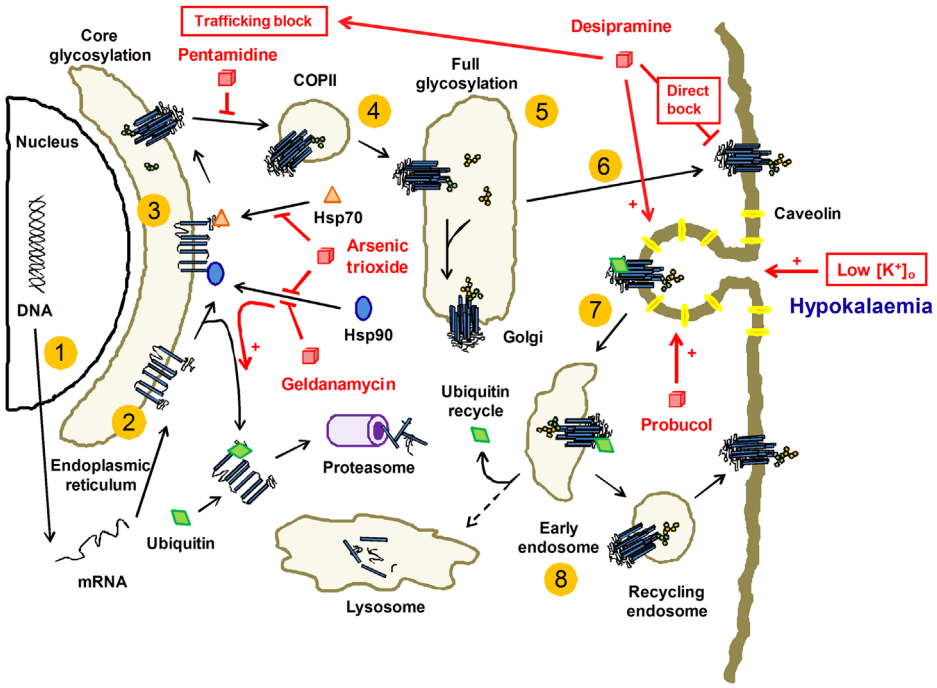

Fig. 1. Schematic diagram of hERG channel protein trafficking and mechanism of drug-induced inhibition of hERG expression in the plasma membrane. The hERG mRNA is processed in the nucleus (1), and then acts to synthesise nascent polypeptide (2). The polypeptide chains are associated with heat shock protein Hsp70 and Hsp90 in endoplasmic reticulum (ER) (3). The still-immature channel is trafficked from the ER to the Golgi by COPII (4), where it is fully glycosylated to form a mature and stable conformation (5). The fully mature hERG channel is transported to the plasma membrane (6). The hERG channel protein of surface expression is tagged with covalently bound ubiquitin and then internalised by caveolin-dependent endocytosis (7). The internalised channels are degraded either via the proteasomal or lysosomal pathway, or recycled via recycling endosomes (8). Arsenic trioxide reduces the formation of hERG–chaperone (Hsp90 and Hsp70) complexes. Geldanamycin accelerates hERG channel degradation by inhibiting the formation of hERG–Hsp90 complexes. Pentamidine inhibits hERG trafficking from the ER without interference with chaperones. Probucol accelerates the degradation of mature hERG channels from the cell membrane through accelerated caveolin-1 turnover. Desipramine exerts multiple effects on hERG; direct channel blockade, disruption of hERG trafficking and degradation due to channel ubiquitination. See text for details (Sections 2 and 3).

COPII (Lee et al., 2004; Delisle et al., 2009), where it is fully glycosylated to form a mature and stable conformation (Ficker et al., 2003). The ER-to-Golgi transition is the rate-limiting step (Sun et al., 2004) and the 135-kDa core-glycosylated immature form in the ER and 155-kDa fully glycosylated mature form in the plasma membrane can be distinguished by molecular weight (Zhou et al., 1998). Finally, the fully mature hERG channel is transported to the plasma membrane. The density of surface expression of functional hERG channels is determined by fine balance between the transport of channels to the plasma membrane and their degradation, and was shown to decay with a half-life of about 11 h (Steele et al., 2007; Ficker et al., 2003). In the clathrin-independent pathway, destabilised hERG protein is tagged with covalently bound ubiquitin and then internalised by caveolin-dependent endocytosis from caveolae, which are specialised lipid rafts with a flask-shaped morphology. The internalised channels are degraded either via the proteasomal or lysosomal pathway, or recycled via recycling endosomes (Gong et al., 2005; Chapman et al., 2005; Sun et al., 2011).

3. Mechanisms of drug-induced inhibition of hERG trafficking

Arsenic trioxide used in the treatment of acute promyelocytic leukaemia was reported to cause QT interval prolongation and TdP (Unnikrishnan et al., 2001), but its mechanism of cardiac toxicity remained unclear. Ficker et al. (2004) demonstrated that arsenic trioxide did not show any direct inhibitory effect on hERG channel activity, whereas long-term exposure of hERG-expressing cell lines and ventricular myocytes to arsenic trioxide reduced hERG channel expression in the plasma membrane and prolonged the duration of cardiac action potential at clinically relevant concentrations. These observations added new insights into the mechanism of hERG inhibition in drug-induced acquired long QT syndrome. To date, several drugs that are known to cause QT prolongation and cardiac arrhythmia in clinical settings were also reported to decrease the surface expression of hERG channel by various mechanisms (Fig. 1). Arsenic trioxide was shown to reduce the formation of hERG–chaperone (Hsp90 and Hsp70) complexes, resulting in disruption of hERG trafficking (Ficker et al., 2004). A specific inhibitor of Hsp90, the antibiotic geldanamycin (Whitesell et al., 1994) accelerates hERG channel degradation by inhibiting the formation of hERG–Hsp90 complexes by blockade of the functionally important ATP activity of Hsp90 (Ficker et al., 2003). In contrast, the antiprotozoal agent pentamidine, which has no direct hERG-blocking activity, inhibits hERG trafficking without interference with chaperones. Pentamidine binds to hERG protein in a folding intermediate conformation leading to arrest of channel maturation and disrupted transport from the ER (Dennis et al., 2012; Varkevisser et al., 2013). Its inhibitory effect is reversed in the presence of pharmacological chaperones, astemizole and dofetilide, suggesting that pentamidine and pharmacological chaperones compete for the same binding site within the hERG channel, although the precise mechanism of action of pentamidine has not been elucidated.

In contrast to forward trafficking inhibition, probucol, a cholesterol-lowering drug, accelerates the degradation of mature hERG channels from the cell membrane through accelerated caveolin-1 turnover (Guo et al., 2011). Caveolin and cholesterol are important components of caveolae that are involved in clathrin-independent endocytosis (Hansen and Nichols, 2009). Furthermore, the caveolin-dependent endocytosis of hERG is also accelerated under conditions of low extracellular K^+ concentration ($[K^+]_o$), clinically known as hypokalaemia (Massaeli et al., 2010). This degradation process is important